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BIOLOGICAL VARIATIONS IN SOIL PLOTS AS SHOWN BY DIFFERENT METHODS OF SAMPLING[1]

By

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INTRODUCTION

Since it is a well known fact that soil samples taken from different parts of a plot show marked chemical variations, it becomes of interest to know whether greater care should be taken in sampling for biological purposes, and in addition to determine the effect that the method of sampling has on variations. To obtain some information with regard to these points the following work was undertaken.

Two plots, each 1/20 acre in size, and as nearly uniform as it was possible to obtain, were selected. One soil was a heavy clay in timothy sod and the other a sandy loam which had grown a crop of corn during the summer. Samples from these two plots were taken both according to the Brown (1) method and by means of Lipman's (3) sampling tube as shown in figure 1. Brown's method consists of removing the surface inch of soil by means of a sterile spatula from an area 20 inches square, stirring the soil in that area to a depth of 5⅔ inches, placing the soil in sterile paper bags and removing to the laboratory. Samples were taken at points 1, 2 and 3 as shown in figure 2. In the case of the sampling tube, individual samples were taken at points 1, 2 and 3, then composite samples were made from the points shown in figure 2. The necessary care was taken to prevent any soil being carried from one point to the next.

Two samplings were made, the first on October 24, 1916, and the second on November 14, 1916. The bacteriological determinations for the first sampling consisted of bacterial counts, and the ammonifying power of the plots was tested with dried blood and cottonseed meal as sources of organic matter. At the later sampling, numbers and the ammonifying power of the plots, with the use of dried blood and peptone, were determined.

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First Sampling

Numbers of Bacteria

As a medium for the determination of numbers, Lipman and Brown's (4) synthetic agar was selected.

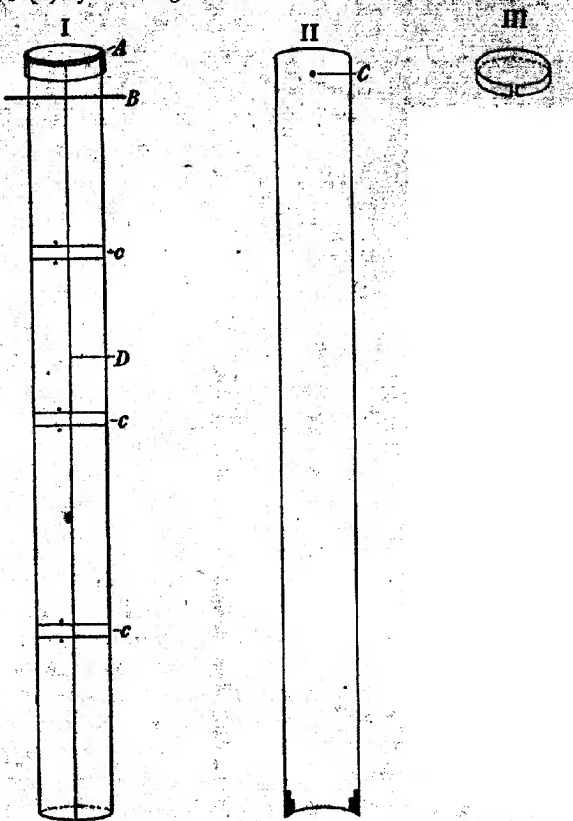

Fig. 1.—I: *a*, Steel Cup; *b*, Steel rod passing through tube; *c*, Rings to hold the two halve together; *d*, line showing where the two halves are joined together.
II: Diagrams of one-half of the sampling tube; *c*, Place where the steel rod passes through.
III: One of the brass rings used to hold the tube together.
(After Lipman, J. G. (3, p. 214).

Although the averages of the plates from any one sample within the plot showed marked deviations with the different methods of sampling, when the general average was taken as the mean, the deviation between

parallel plates was often greater than the deviation from the mean. Great care was exercised in pouring the plates, but nevertheless the results were unsatisfactory and are not recorded. Rapid growth of fungi was responsible in many cases for poor results. It is hoped that future work will show more favorable results.

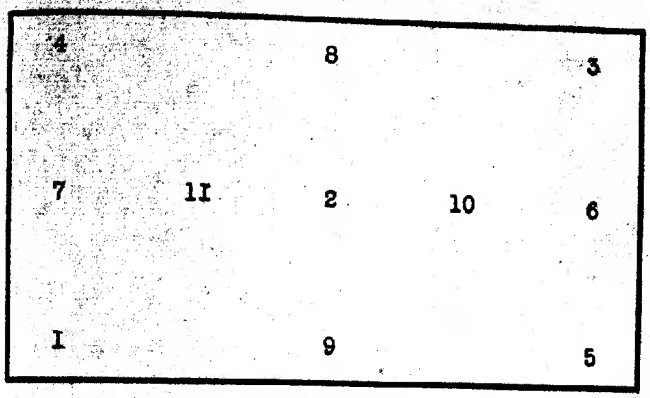

Fig. 2.—Diagram showing the points where samples were taken.

Ammonification of Dried Blood

The fresh-soil method, involving the use of dried blood equivalent to 100 mg. of nitrogen and analyzing 12.48 per cent nitrogen, was employed. The soil shaker (2) was used for mixing the organic matter with the soil and was sterilized by means of ignited alcohol whenever a different soil sample was used. The soils were at optimum moisture content when sampled, therefore no additional water was added. The incubation period was 6 days at 20° C. The ammonia produced was determined by the magnesium oxide method. The results are recorded in Table I.

The data show that in general Brown's method gives higher results than was obtained with the sampling tube. With this method the deviation from the mean for the meadow soil is approximately 10 per cent as contrasted with about 4 per cent for the sampling tubes. Considering the range of sampling in the meadow soil, it will be seen that the deviation is not marked under either method, the deviation between parallels in general not being greater than the deviation from the mean. In the sandy soil there is no appreciable difference between the results from the samples obtained by the different methods, the ammonifying power

TABLE I

THE INFLUENCE OF THE RANGE AND METHODS OF SAMPLING UPON THE AMMONIFYING POWERS OF VARIOUS SOILS: DRIED BLOOD AS THE SOURCE OF ORGANIC MATTER

Soil Sampled	Range of Sampling	Ammonia Accumulation			Ave.	Mean	Deviation from Mean	Greatest Deviation between Parallels
		Mg. N	Mg. N	Mg. N	Mg. N			
Timothy Meadow. Alloway Silt Loam. N. J. Agr. Exp. Sta.	Single core 1	26.10	26.05	28.10	26.75	27.61	0.14	0.05
	Single core 2	26.86	27.43	26.42	26.90		—0.47	1.01
	Single core 3	23.67	23.29	25.43	24.13		—0.47	2.14
	Composite of 3 cores	27.62	27.62	27.71	27.65		+0.26	0.09
	Composite of 5 cores	28.24	26.58	25.95	26.92		—0.47	0.66
	Composite of 11 cores	28.24	27.32	27.52	27.36		—0.25	0.92
	Brown's method 1	30.86	31.43	30.25	30.84		+3.45	1.19
	Brown's method 2	28.48	27.44	26.68	27.53		—0.14	0.96
	Brown's method 3	30.52	30.81	30.05	30.46		+3.05	0.76
Cultivated Plot 49. Sassafras Sandy Loam. N. J. Agr. Exp. Sta.	Single core 1	32.91	32.64	32.44	32.66	28.20	+4.46	0.47
	Single core 2	28.25	27.06	27.62	27.64		—0.56	1.19
	Single core 3	27.38	26.95	25.60	26.64		—1.56	1.78
	Composite of 3 cores	27.05	28.05	26.95	27.35		—0.85	1.10
	Composite of 5 cores	*22.11	25.95	26.62	26.28		—1.98	0.67
	Composite of 11 cores	26.40	27.81	27.24	27.15		—1.05	1.41
	Brown's method 1	32.24	31.00	31.95	31.73		+3.53	1.24
	Brown's bethod 2	27.35	27.15	*23.27	27.25		—1.00	0.30
	Brown's method 3	27.81	26.20	27.43	27.14		—1.06	1.61

* Omitted from average.

TABLE II

THE INFLUENCE OF THE RANGE AND METHODS OF SAMPLING UPON THE AMMONIFYING POWERS OF VARIOUS SOILS: COTTONSEED MEAL AS THE SOURCE OF ORGANIC MATTER

Soil Sampled	Range of Sampling	Ammonia Accumulation			Ave.	Mean	Deviation from Mean	Greatest Deviation between Parallels
		Mg. N	Mg. N	Mg. N	Mg. N			
Timothy Meadow. Alloway Silt Loam. N. J. Agr. Exp. Sta.	Single core 1	26.33	27.10	*23.52	26.71	29.79	—3.08	0.77
	Single core 2	30.43	28.57	29.39	29.46		—0.33	1.86
	Single core 3	31.43	31.32	*29.81	31.37		+1.58	0.11
	Composite of 3 cores	27.49	32.32	30.06	29.96		+0.17	4.83
	Composite of 5 cores	29.29	30.86	30.24	30.13		+0.34	1.57
	Composite of 11 cores	29.43	28.53	29.57	29.17		—0.62	1.04
	Brown's method 1	29.62	29.52	29.62	29.59		—0.20	0.10
	Brown's method 2	29.43	28.58	28.29	28.76		—1.03	1.14
	Brown's method 3	33.67	33.05	32.37	33.03		+3.24	1.30
Cultivated Plot 49. Sassafras Sandy Loam. N. J. Agr. Exp. Sta.	Single core 1	40.95	40.63	40.77	40.78	37.92	+2.86	0.32
	Single core 2	38.05	37.72	38.48	38.08		—0.16	0.76
	Single core 3	36.05	36.24	35.72	36.00		—1.92	0.52
	Composite of 3 cores	lost	37.62	37.50	37.56		—0.36	0.12
	Composite of 5 cores	37.34	37.52	37.14	37.33		—0.59	0.38
	Composite of 11 cores	36.67	36.82	38.24	37.24		—0.68	1.57
	Brown's method 1	37.72	39.81	39.52	39.01		+2.09	2.09
	Brown's method 2	38.62	38.62	*34.47	38.62		+0.70	0.00
	Brown's method 3	37.62	35.92	36.58	36.70		+1.22	1.70

* Omitted from average.

in both cases being practically the same. In regard to the range of sampling the ammonifying power throughout the plot is practically the same with one exception, point 1 having a higher ammonifying power as shown by both methods.

Ammonification of Cottonseed Meal

The method employed was the same as that used in the case of dried blood except for the different source of organic matter. Table II gives the data obtained.

With cottonseed meal as a source of energy the same general tendency holds true as was found with dried blood. However, there are some changes to note in the case of the timothy meadow. Whereas with dried blood there was a low ammonification at point 3, with cottonseed meal there is a very high ammonification. Also, sample 3 of Brown's method records the greatest ammonia accumulation of all samplings. Again the range of sampling shows no wide deviations, the ammonia accumulation being practically the same with one or two exceptions; that is to say, at point 1 for the tube sampling and point 3 for the Brown method as mentioned above.

Second Sampling

The bacteriological determinations for the second sampling were carried on in the same manner as for the first sampling with the exception that peptone solution was substituted for cottonseed meal in the hope that this material would show greater differences.

Numbers of Bacteria

The data resulting from the bacterial counts, although better than at the previous sampling, were again unsatisfactory and are not tabulated.

Ammonification of Dried Blood

During the time elapsing between the first and second samplings there had been no rain; consequently, the soil moisture was below optimum and water was added to the tumblers. Comparing the two methods (Table III) we find that in the meadow soil Brown's method in 2 out of 3 cases gives about 10 per cent higher ammonifying efficiency than the other method. With regard to the range of sampling, considering the tube method, there is practically no difference, with one exception (point 2). In general, in the sandy soil there are no great differences in ammonification between the samples obtained by the different methods and of different range, except at point 1, which, it will be remembered, was high at the previous sampling.

Ammonification of Peptone

One hundred c.c. of a 1 per cent peptone solution, containing 50 mg. of K_2HPO_4 per liter were inoculated with 5 c.c. of a 1 to 5 soil infusion,

TABLE III

THE INFLUENCE OF THE RANGE AND METHODS OF SAMPLING UPON THE AMMONIFYING POWERS OF VARIOUS PLOTS: SECOND SAMPLING—DRIED BLOOD AS THE SOURCE OF ORGANIC MATTER

Soil Sampled	Range of Sampling	Ammonia Accumulation Mg. N	Mg. N	Mg. N	Ave. Mg. N	Mean	Deviation from Mean	Greatest Deviation between Parallels
Timothy Meadow. Alloway Silt Loam. N. J. Agr. Exp. Sta.	Single core 1	37.32	38.17	37.18	37.56	40.03	—2.46	0.99
	Single core 2	45.61	48.90	47.61	47.37		+7.35	3.29
	Single core 3	35.89	36.40	37.46	36.58		—3.46	1.57
	Composite of 3 cores	37.32	36.40	39.32	37.68		—2.34	2.92
	Composite of 5 cores	lost	35.22	37.32	36.27		—3.29	2.10
	Composite of 11 cores	40.18	39.56	40.32	40.02		0.00	0.76
	Brown's method 1	42.75	42.75	42.90	42.80		+2.78	0.15
	Brown's method 2	44.61	44.18	44.33	44.37		+4.35	0.43
	Brown's method 3	37.89	37.18	lost	37.53		—2.49	0.71
Cultivated Plot 49. Sassafras Sandy Loam. N. J. Agr. Exp. Sta.	Single core 1	40.61	43.70	42.61	42.30	36.99	+5.31	3.09
	Single core 2	34.89	32.32	33.17	33.46		—3.53	2.57
	Single core 3	35.02	36.32	35.75	35.69		—1.30	1.30
	Composite of 3 cores	37.39	37.03	36.32	36.91		—0.08	1.07
	Composite of 5 cores	34.03	34.74	34.32	34.36		—2.63	0.71
	Composite of 11 cores	32.35	33.74	33.67	33.25		—3.74	1.39
	Brown's method 1	43.75	43.75	43.47	43.64		+6.65	0.28
	Brown's method 2	37.18	35.75	lost	36.46		—0.53	1.43
	Brown's method 3	36.03	37.24	37.24	36.83		—0.16	1.21

TABLE IV

THE INFLUENCE OF THE RANGE AND METHODS OF SAMPLING UPON THE AMMONIFYING POWERS OF VARIOUS SOILS: SECOND SAMPLING—THE AMMONIFICATION OF PEPTONE

Soil Sampled	Range of Sampling	Ammonia Accumulation Mg. N	Mg. N	Mg. N	Ave. Mg. N	Mean	Deviation from Mean	Greatest Deviation between Parallels
Timothy Meadow. Alloway Silt Loam. N. J. Agr. Exp. Sta.	Single core 1	26.50	26.50	26.50	26.50	26.46	+0.04	0.00
	Single core 2	25.60	26.70	26.50	26.27		—0.19	1.10
	Single core 3	29.60	29.20	29.70	29.50		+3.04	0.50
	Composite of 3 cores	24.70	24.70	25.70	25.03		—1.03	1.00
	Composite of 5 cores	25.70	26.60	26.50	26.26		—0.20	0.90
	Composite of 11 cores	29.30	28.40	30.20	29.30		+2.84	1.80
	Brown's method 1	24.50	24.70	24.20	24.46		—2.00	0.50
	Brown's method 2	23.60	24.70	24.60	24.30		—2.16	1.10
	Brown's method 3	26.70	*28.90	26.45	26.57		+0.11	0.25
Cultivated Plot 49. Sassafras Sandy Loam. N. J. Agr. Exp. Sta.	Single core 1	23.40	23.90	25.00	24.10		.....	.....
	Single core 2	26.90	24.60	24.70	25.40		.....	.....
	Single core 3	26.90	28.90	*19.10	27.90		.....	.....
	Composite of 3 cores	16.50	17.90	17.80	17.40	16.33	+1.07	1.40
	Composite of 5 cores	14.50	15.50	15.10	15.03		—1.30	1.00
	Composite of 11 cores	13.00	16.50	15.70	15.06		—1.27	3.50
	Brown's method 1	19.00	16.40	15.60	17.00		+0.67	3.40
	Brown's method 2	17.65	18.00	18.40	18.01		+1.67	0.75
	Brown's method 3	15.30	14.20	17.00	15.50		—0.83	2.80

* Omitted from average.

incubated for 3 days at 20° C. and the ammonia determined by the magnesium oxide method. The results are given in Table IV.

In the case of the meadow soil there is little difference shown between the two methods or in the range of sampling, with the exception of point 3, which gave high results with both methods. It will be remembered that point 3 also gave high results with cottonseed meal at the first sampling. In the sandy soil the single cores all ran very high, whereas the composite cores showed lower ammonification. Brown's method also gave results comparable with the composite samples taken by the tube method. Excluding single cores 1, 2 and 3, which were decidedly abnormal, the deviations between samples by both methods are not large

Summary

A resumé of the above data would seem to suggest that where plots are uniform in character the biological variations of the soil at different points in the plot are not great, or else we are not able to detect these differences by the present methods both for ease of taking the sample and from the standpoint of destruction of the plot, especially in an uncultivated area.

The tube method is superior to Brown's method.

Literature Cited

(1) Brown, P. E.
1912. Bacteriological studies of field soils; II. Iowa Agr. Exp. Sta. Research Bul. 6, p. 221.

(2) Lint, H. C., and Coleman, D. A.
1916. Sources of error in soil bacteriological analysis. *In* Soil Sci., v. 2, no. 2, p. 157-162.

(3) Lipman, J. G.
1901. Report of the assistant in soil chemistry and bacteriology. *In* N. J. Agr. Exp. Sta. 22nd Ann. Rpt., p. 213-215.

(4) Lipman, J. G., and Brown, P. E.
1908. Notes on methods and culture media. *In* N. J. Agr. Exp. Sta. 29th Ann. Rpt., p. 132.

THE EFFECT OF AMMONIUM SULFATE ON SOIL ACIDITY[1]

By

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Introduction

It is a well known fact that soils on which ammonium sulfate is used year after year increase in acidity much more rapidly than untreated soils, and in extreme cases the acid production may be great enough to prevent all crop growth. The purpose of this experiment was to measure the increase on various types of soil and to determine the effect of removing a portion of the added nitrogen by means of crops.

Experimental

In addition to a nearly pure quartz sand, 5 field soils were selected. These are classified as Norfolk sand, Collington sandy loam, Sassafras loam, Penn loam and Alloway clay, all acid except the Collington sandy loam. The Norfolk sand was low in organic matter and rather coarse in texture. The Collington sandy loam contained little fine material but was made up chiefly of greensand marl and sand. The Sassafras loam was a soil of medium texture and fertility. The Penn loam was similar to the Sassafras except that it was somewhat heavier and more fertile. The Alloway clay was a stiff soil containing more clay particles than any of the other soils used but was not quite as heavy as the Penn loam. The lime-requirement, water-holding capacity, hygroscopic moisture and apparent specific gravity of each of the soils used is given in Table I. The water-holding capacity is a fair index of the texture of the various soils.

For the carrying out of this experiment small pots, capable of holding 2.5 pounds of soil, were used. Four pots of each soil were weighed out and fertilized with 1 gm. each of acid phosphate and potassium sulfate. Half of the pots received ammonium sulfate at the rate of 0.5 gm. per pot, one of each of these treated and untreated pots being planted to

[1] Received for publication December 16, 1916.

buckwheat, and the other two left uncropped. At the end of every 3 months, 0.5 gm. additional ammonium sulfate was added, until a total of 2 gm. had been applied and 4 crops grown. The crops were always harvested as the seeds were ripening and analyzed for nitrogen. The total yields for the 4 crops of buckwheat and the nitrogen recovered in the various soils are given in Table II.

TABLE I

PROPERTIES OF THE SOILS USED

	Quartz Sand	Norfolk Sand	Collington Sandy Loam	Sassafras Loam	Penn Loam	Alloway Clay
Hygroscopic moisture, per cent....	0.10	0.15	1.60	1.31	2.28	0.88
Apparent specific gravity	1.58	1.52	1.34	1.18	1.10	1.30
Water-holding capacity, per cent..	30.00	34.00	42.00	49.00	53.00	44.00
Lime-requirement as pounds CaO per 3,000,000 pounds of soil....	350	825	Alkaline	2100	2250	1800

The yields for the individual crops, which it seemed unnecessary to include in this paper, show a vigorous growth for the first crop, much smaller for the second and practically no growth at all for some of the soils in the case of the third and fourth crops. This is due to an ex-

TABLE II

SUMMARY OF THE YIELDS OF BUCKWHEAT AND THE NITROGEN RECOVERED IN VARIOUS SOILS CROPPED CONTINUOUSLY FOR ONE YEAR

Pot No.	Soils Used	Ammonium Sulfate Added	Yield gm.	Increase Over Check	Total Mg. N	Increase Mg. N	Per cent Recovery
1	Quartz sand	0	1.2	...	18.6	....	..
2	Quartz sand	416	0	—1.2	0	—18.6	0
3	Norfolk sand	0	5.8	...	41.5	....	..
4	Norfolk sand	416	15.7	9.9	197.4	155.9	37.5
5	Collington sandy loam	0	4.0	...	36.0	....	..
6	Collington sandy loam	416	10.8	6.8	141.6	105.6	25.4
7	Sassafras loam	0	4.3	...	38.7	....	..
8	Sassafras loam	416	12.3	8.0	195.4	156.7	37.6
9	Penn loam	0	6.7	...	56.1	....	..
10	Penn loam	416	15.3	8.6	353.6	297.5	71.5
11	Alloway clay	0	6.7	...	53.7	....	..
12	Alloway clay	416	14.2	7.5	245.0	191.3	45.9

haustion of the available plant-food in the check pots and to too much ammonium sulfate in those soils receiving this fertilizer. The quantities of nitrogen applied were very large for such small amounts of soil. The first application of ammonium sulfate to the quartz sand was sufficient to prevent the growth of the buckwheat. The analyses for nitrogen in the various crops show that in the presence of very high acidity and large

quantities of ammonium sulfate the nitrogen in the buckwheat is increased several times above the amount normally present. The percentage recovery of nitrogen, as given in Table II, is large regardless of the fact that during the last half of the experiment the crop yields were small.

TABLE III

INCREASES IN ACIDITY ON CROPPED SOILS

Pot No.	Soils Used	Mg. N Added	N Removed in Crop	Ammonium Sulfate N Remaining	Initial Acidity as pounds CaO	Acidity at End of Experiment	Increase in Acidity	Increase Due to Ammonium Sulfate
1	Quartz sand	0	18.6	.....	350	900	550	....
2	Quartz sand	416	0	416.0	350	2500	2150	1600
3	Norfolk sand	0	41.5	.....	825	7900	7075	....
4	Norfolk sand	416	197.4	260.1	825	12200	11375	4300
5	Collington sandy loam	0	36.0	.....	Alkaline	4600	4600	....
6	Collington sandy loam	416	141.6	310.4	Alkaline	8900	8900	4300
7	Sassafras loam	0	38.7	.....	2100	4800	2700	....
8	Sassafras loam	416	195.4	259.3	2100	8900	6800	4100
9	Penn loam	0	56.1	.....	2250	10200	7950	....
10	Penn loam	416	353.6	118.5	2250	15600	13450	5500
11	Alloway clay	0	53.7	.....	1800	8200	6400	....
12	Alloway clay	416	245.0	224.7	1800	11200	9400	3000

TABLE IV

INCREASES IN ACIDITY ON UNCROPPED SOILS

Pot No.	Soils Used	Mg. N Added	Initial Acidity as pounds CaO	Acidity at End of Experiment	Increase in Acidity	Increase Due to Ammonium Sulfate
13	Quartz sand	0	350	900	550	....
14	Quartz sand	416	350	3100	2750	2200
15	Norfolk sand	0	825	7600	6775	....
16	Norfolk sand	416	825	12200	11375	4600
17	Collington sandy loam	0	Alkaline	4200	4200	....
18	Collington sandy loam	416	Alkaline	8100	8100	3900
19	Sassafras loam	0	2100	4200	2100	....
20	Sassafras loam	416	2100	8300	6200	4100
21	Penn loam	0	2250	10600	8350	....
22	Penn loam	416	2250	14200	12050	3700
23	Alloway clay	0	1800	7800	6000	....
24	Alloway clay	416	1800	12200	10400	4400

After the last crop had been harvested the soils were removed from the pots, thoroughly mixed, air-dried and the lime-requirements determined by the Veitch method. The increases in acidity on the cropped and uncropped soils are given in Tables III and IV, respectively, and shown diagramatically in figure 1.

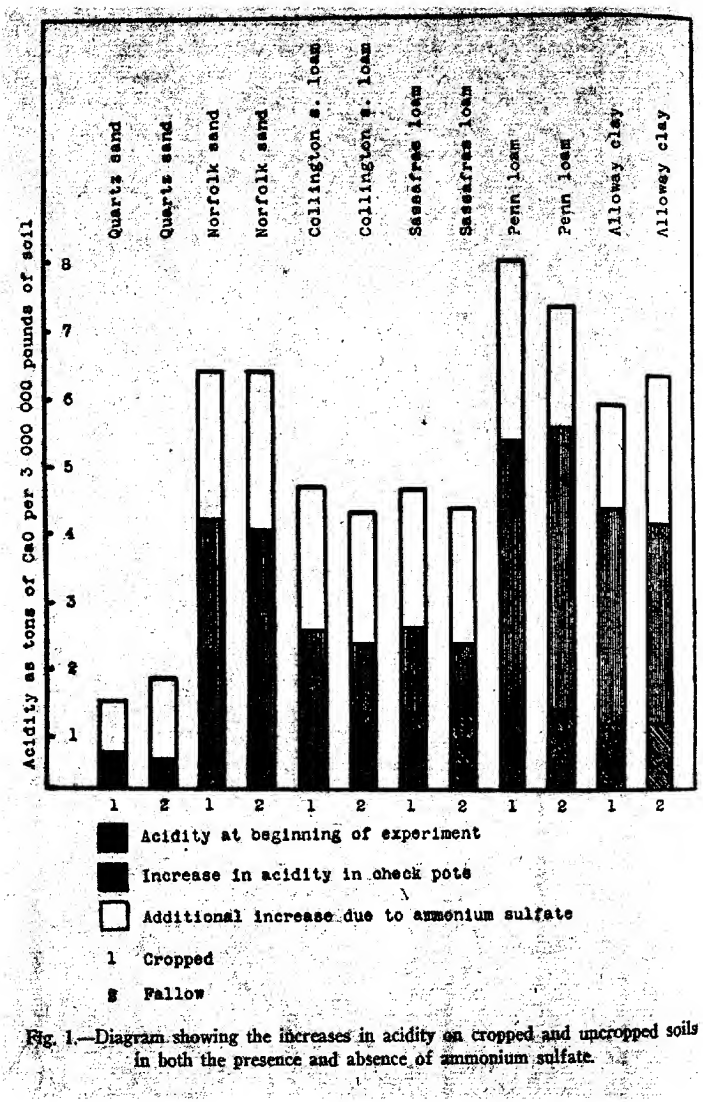

Fig. 1.—Diagram showing the increases in acidity on cropped and uncropped soils in both the presence and absence of ammonium sulfate.

In both the check pots and those receiving ammonium sulfate there was a very decided increase in acidity during the year in the greenhouse. The increases in the cropped pots receiving no nitrogen varied from 550 pounds of CaO per 3,000,000 pounds of soil in the case of the quartz sand to 7,950 pounds for the Penn loam. Similarly, for the uncropped soils the increases varied from 550 pounds for the quartz sand to 8,350 pounds for the Penn loam. It is significant that the Penn loam, which was the most acid soil at the beginning of the experiment, also shows the greatest increase in acidity. A comparison of the soils from the standpoint of increases in acidity in the check pots shows little difference between those which were cropped and the ones kept in fallow. The greatest increase in acidity was obtained with the soils in the following order: Penn loam, Norfolk sand, Alloway clay, Collington sandy loam, Sassafras loam, and quartz sand.

All soils showed a marked increase in acidity due to ammonium sulfate, varying from 2,200 pounds for the uncropped quartz sand to 4,600 pounds for the Norfolk sand without a crop. Where buckwheat was grown, quartz sand showed the lowest increase, amounting to 1,600 pounds, and the Penn loam the highest, namely, 5,500 pounds. Exclusive of the quartz sand, ammonium sulfate caused a fairly uniform increase in acidity in all soils, the average being 4,240 pounds where a crop was grown, and 4,140 pounds in the absence of a crop. Two soils showed the largest increases where a crop was grown, three in the uncropped pots and one showed no difference. Generally speaking, the removal of a portion of the added nitrogen by the buckwheat had no marked effect upon the accumulation of acidity.

If the increase in lime-requirement in the pot experiment is figured on the basis of a 100-pound application of ammonium sulfate, then approximately 80 pounds of calcium oxide, or 140 pounds of calcium carbonate are required to neutralize the acid produced. The variations that might be expected from this mean for different types of soil have already been discussed and shown diagramatically in figure 1.

Summary

With due regard for the limitations of the experiment, namely single treatment, the following points are advanced.

1. The increases in acidity in 5 greenhouse soils and a quartz sand, receiving no nitrogenous fertilizer, were practically the same during the course of a year whether these soils were cropped or kept in fallow. The quartz sand showed the smallest increase in acidity and a loam soil the largest, but there was no relation between the acid accumulation and the soil texture.

2. The increases in acidity in the presence of ammonium sulfate were markedly higher than in the check pots. The partial removal of the nitrogen added decreased the acidity to an appreciable extent in the quartz sand and in the heavy clay soil, increased it in the loam, and left it practically the same in the other three soils. The average increase in acidity in the soils used, exclusive of the quartz sand, was 4,140 pounds of calcium oxide per 3,000,000 pounds of soil where no crop was grown, and 4,240 pounds where 4 crops of buckwheat were harvested.

3. On the average, the increase in acidity produced by ammonium sulfate in greenhouse pots was about 80 pounds of calcium oxide for 100 pounds of ammonium sulfate applied.

CLAY BOULDERS AND THE ROLLING ACTION OF WATER[1]

By

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An interesting and rather unusual display of clay boulders was recently noticed on the upper part of the large Huenerne fan in Ventura County, California. This plain is bordered on the east, in places, by well developed old terraces or elevated beaches. The terraces have been elevated and have remained in their present position so long that a subsoil considerably heavier in texture than the surface soil has been developed. The surface soil varies from a loam to a fine sandy loam. It is friable and quite permeable to water. The subsoil is a clay loam and relatively impermeable to water. This produces ideal conditions for erosion, a permeable surface and a less permeable subsoil, and the result is that erosion on these terraces is very severe.

During December, 1916, a great deal of rain fell and it culminated in a rain of at least 2.5 inches on December 24. The outwash from these terraces spread over the fan for a considerable distance and covered several square miles with a fresh deposit which varied in texture from fine gravel and sand, dropped near the source, to very fine sand and silt and even to clay on the extreme outer edge of the wash.

The clay boulders were noticed at several places, but always at a considerable distance from their source. A good display was observed a short distance northwest of Springville, where these boulders had been left near the road. The boulders were from 3 inches to 12 and 15 inches in diameter. They were rounded, having the typical shape of water-worn stones. In texture they were clay loam, and not clay, but it seems best to retain the name "clay boulders." Many gravel stones averaging about 1 inch in diameter were imbedded in the surface. These stones had evidently been picked up on their journey, for the interior of the boulders contained none. The boulders had unquestionably come from the clay loam subsoil of the terraces to the northeast, and must have traveled at least a mile and perhaps more, for it is impossible to say what part of the hills they started from. This is a considerable distance to travel in one day, for the flood which moved them was of only a few hours duration. The alluvium left by this same flood, in the neighborhood of the boulders, was a fine sandy loam. This had been carried in suspension by the water and

[1] Received for publication January 25, 1917.

deposited here because of decreased velocity of the current. But the boulders were never in suspension. They were rolled into place. This is a fine illustration of the two independent actions of running water, the carrying of articles in suspension, and the rolling of larger particles along its bed.

I have seen coarse sand which has been deposited on the Mississippi River bottom through a crevasse in the levee below Natchez. This sand came from the bed of the river and its origin was many hundred miles to the north. It had been rolled along the river bed, probably quite intermittently. Finally, on account of the crevasse in the levee during flood times, the current was strong enough to pick it up and deposit it over the silts and clays which had previously been deposited. This is one of the reasons why alluvial soils are so hard to map. The forces that put them into place are very complicated.

But the distinction between the carrying and the rolling properties of running water has been too little recognized by those who study the forces that make soils. The carrying action of water is frequently discussed in relation to the building of fans and flood plains, but the discussion generally stops there and no mention is made of the material rolled along the bed of the stream, or, as in this case, rolled over the surface of the fan by the swiftly rushing water of floods.

The flood plain of the Merced River, near Snelling, California, has two distinct strata. The surface soil is a silt loam and across the whole extent of the flood plain which is several miles wide, and up and down the valley for miles, the texture is the same. It is a remarkably uniform piece of soil. Lying under this soil at varying depths, usually from 3 to 6 feet, and occasionally coming to the surface, is a stratum of cobble. The noteworthy feature is the absence of soil particles of intermediate grade between the fine soil and the coarse cobble. They do not grade into each other. The only satisfactory explanation of the way in which these two deposits were laid down, as far as the writer can see, is that the silt loam was deposited from the material suspended in the water and the cobble was rolled into position. The river in this valley swings from one side of its flood plain to the other, and as it deserts an old channel which is filled with cobble rolled down from the mountains above an opportunity is given for the deposition of the silt, for the waters now going into the old channel during floods are either very sluggish or stagnant.

IS THE HUMUS CONTENT OF THE SOIL A GUIDE TO FERTILITY?[1]

By

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Introduction

In recent years there has been a tendency on the part of numerous investigators to question the value of the humus determination in soil fertility work. In the Journal of the American Chemical Society (5) is found the following paragraph from the Iowa Agricultural Experiment Station: "The organic matter extracted by alkali is of no very different character than the organic matter of the soil as a whole. This, together with the fact proved by Fraps and Hammer (2), that upon adding organic matter to the soil at the end of a year's time there is no more material extracted with diluted ammonia than at the beginning of the period, proves quite conclusively that the determination of the amount of humus as found by the different methods is of no particular value in the study of a soil." Investigations by Gortner (3) led him to conclude that there is no increase in ammonia-soluble humus after a year of humification. In other investigations by Weir (7) on soil extracted with sodium hydroxide, he found that, "Approximately equal total yields, both of dry matter and nitrogen, were obtained over four successive crops. It thus appears that the removal of soluble humus had no effect of diminishing the productiveness of the soil in spite of the fact that the soil used was known to respond to nitrogenous fertilizers."

Humification Problems

The writer has been studying the effect of humification of various farm manures as well as green manures and has made vegetative tests to aid in estimating availability of plant-food contained in the complex plant molecules. The growing plant cannot untilize directly much of the plant-food contained in other plant products until certain rearangements in the molecules have taken place. This is usually brought about by bacterial and weathering agencies cleaving off certain fractions, probably in the order of their availability to the growing plant.

[1] Received for publication May 16, 1917.

Work done recently in this laboratory shows that 25 per cent of the nitrogen in a loam soil containing a total of 4700 pounds of nitrogen per acre was insoluble in 20 per cent HCl after 120 hours' digesting on a steam bath; also, that 20 per cent of the nitrogen in ground alfalfa was insoluble in 20 per cent HCl after 40 hours' boiling. Again, Brigham (1) has shown that linseed and cottonseed meals are largely unavailable to growing corn until broken down by *Bacillus subtilis* and certain cleavage products liberated from the molecule.

Methods Employed

An effort was made to measure the rate of cleavage by determining the per cent present and the rapidity of increase of humus in the soil as measured by its ammonia-soluble organic matter, by the method devised by Grandeau (4), modified by Smith (6). An attempt was made, also to measure the amount of humus by a colormetric method, but this was given up because there seems to be but little relationship between the amount of humus present and the degree of color of the ammonia-soluble matter. The color is probably due to the presence of pigments and soluble salts and is not directly related to humus content.

Plan Followed

A clay surface soil was used which was very deficient in organic matter. The soil was screened and mixed with different manures, then placed in double boxes holding a cubic foot each and the boxes were buried 8 inches apart in a trench, allowing the top to project a little above the level of the surrounding ground. It was thought that the moisture could be kept more constant in this way. In each box 1 pound of dry matter was used which was equivalent in fresh manure to the weights given in Table 1.

TABLE I

KINDS AND AMOUNTS OF ANIMAL MANURES USED

Box 1	contained 2.0 lbs.	hen manure	plus 50 gm.	$CaCO_3$
Box 2	contained 3.2 lbs.	sheep manure	plus 50 gm.	$CaCO_3$
Box 3	contained 2.4 lbs.	hog manure	plus 50 gm.	$CaCO_3$
Box 4	contained 4.0 lbs.	horse manure	plus 50 gm.	$CaCO_3$
Box 5	contained 6.6 lbs.	steer manure	plus 50 gm.	$CaCO_3$
Box 6	contained 6.0 lbs.	cow manure	plus 50 gm.	$CaCO_3$
Box 7	contained 4.0 lbs.	horse manure	plus 101 gm.	CaOMgO
Box 8	contained 4.0 lbs.	horse manure	plus 171 gm.	CaO
Box 9	contained 4.0 lbs.	horse manure	plus 179 gm.	$CoCO_3MgCO_3$
Box 10	contained 4.0 lbs.	horse manure	plus 175 gm.	$CaCO_3$

The soils were mixed with the manure and buried in the trenches May 30, 1914. Samples were taken from the boxes the same day. Other samples were taken on the following dates: November 25, 1914; February 16, 1915, after the winter freeze; April 13, 1915, after a period of warm spring weather; June 1, 1915, and October 15, 1915.

Humification of Green Manures

On October 10, 1915, a similar experiment was started with the use of green manures instead of animal excrements. The equivalent of 1 pound of dry material was used as before. Table II gives the weights and kinds of green materials used and the methods of applying, the aim of which was to imitate the following farm practices:

1. Turning under a heavy roll of green material.
2. Discing the green mass before plowing.
3. Allowing the material to dry before plowing.
4. Applying 5 tons of ground limestone.

TABLE II

KINDS AND AMOUNTS OF GREEN MANURES USED

Box	1.—Green cowpea stalks (3000 gm.), layer in middle, soil above and below, 100 gm. $CaCO_3$.
Box	2.—Same as Box 1 but not limed.
Box	3.—Green cowpea stalks, chopped up and well mixed with soil.
Box	4.—Green alfalfa (2250 gm.), layer in middle, soil above and below, 100 gm. $CaCO_3$.
Box	5.—Same as Box 4 but not limed.
Box	6.—Green alfalfa chopped up and mixed with soil.
Box	7.—Green sweet clover (2650 gm.), layer in middle, soil above and below, 100 gm. $CaCO_3$.
Box	8.—Same as Box 7 but not limed.
Box	9.—Green sweet clover chopped up and mixed with soil.
Box	10.—Green oat straw (2368 gm.), layer in middle, soil above and below, 100 gm. $CaCO_3$.
Box	11.—Same as Box 10 but not limed.
Box	12.—Green oat straw chopped up and mixed with soil.
Box	13.—Dry cowpea stalks (450 gm.), layer, soil above and below.
Box	14.—Dry alfalfa arranged as in Box 13.
Box	15.—Dry sweet clover as in Box 13.
Box	16.—Dry oat straw as in Box 13.
Box	17.—Soil only (check box).

Humus Determinations

An effort was made to follow some of the changes brought about by bacterial and weathering agencies by determining the per cent of ammonia-soluble material at the time the manures were mixed with the soil and at various subsequent periods.

Green Manures

The percentages of humus at the time the green manures were mixed with the soil and at varying intervals following, depending on the condition of the soil for sampling, are shown in Table V.

Vegetative Test with Green Manures

On May 16, 1916, corn was planted in all of the boxes containing the green manures and on October 1 it was harvested. The results are given in Table VII.

Study of Solubility Changes in Leaves

Some data on changes maple leaves undergo when decomposing have been made recently and will be given in this connection. Leaves were placed in two 500-c.c. Erylemeyer flasks in September 1913, sufficient

water poured upon them to cover the leaves, and the flasks plugged with cotton. One flask was heated about to boiling on three different days,

TABLE III

AVERAGE WEIGHT IN GRAMS OF HUMUS AND ASH IN 1 GRAM OF SOIL

Soils and Manure Unexposed	Hen Manure + 50 gm. $CaCO_3$		Sheep Manure + 50 gm. $CaCO_3$		Pig Manure + 50 gm. $CaCO_3$		Horse Manure + 50 gm. $CaCO_3$		Steer Manure + 50 gm. $CaCO_3$	
	Humus	Ash	Humus	Ash	Humus	Ash	Humus	Ash	Humus	Ash
May 30, 1914	.0057	.0083	.0068	.0080	.0057	.0088	.0058	.0087	.0092	.0076
Nov. 25–Feb. 16	.0069	.0080	.0078	.0065	.0081	.0060	.0061	.0065	.0093	.0076
Feb. 16–Apr. 13	.0073	.0112	.0072	.0100	.0083	.0107	.0063	.0101	.0094	.0100
Apr. 13–June 1	.0078	.0096	.0066	.0096	.0073	.0096	.0068	.0088	.0097	.0098
June 1–Oct. 15	.0071	.0073	.0075	.0087	.0064	.0083	.0083	.0080	.0092	.0094

Check: May 1914. Humus .0049 Ash .0097
Feb. 1915. Humus .0050 Ash .0095

Soils and Manure Unexposed	Cow Manure + 50 gm. $CaCO_3$		Horse Manure + 101 gm. + CaOMgO		Horse Manure + 171 gm. CaO		Horse Manure + 179 gm. $CaCO_3MgCO_3$		Horse Manure + 175 gm. $CaCO_3$	
	Humus	Ash	Humus	Ash	Humus	Ash	Humus	Ash	Humus	Ash
May 30, 1914	.0079	.0087	.0055	.0085	.0048	.0075	.0055	.0075	.0055	.0074
Nov. 25–Feb. 16	.0092	.0074	.0064	.0084	.0051	.0065	.0067	.0087	.0051	.0065
Feb. 16–Apr. 13	.0096	.0115	.0070	.0090	.0061	.0096	.0066	.0099	.0061	.0096
Apr. 13–June 1	.0097	.0102	.0072	.0090	.0058	.0093	.0123	.0062	.0069	.0096
June 1–Oct. 15	.0091	.0086	.0090	.0072	.0081	.0091	.0093	.0097	.0074	.0090

TABLE IV

PER CENT OF GAIN OR LOSS IN HUMUS

Difference between Check and Manure-Soil Mixture when Mixed	Hen Manure + $CaCO_3$	Sheep Manure + $CaCO_3$	Pig Manure + $CaCO_3$	Horse Manure + $CaCO_3$	Steer Manure + $CaCO_3$	Cow Manure + $CaCO_3$	Horse Manure CaOMgO	Horse Manure + CaO	Horse Manure + $CaCO_3MgCO_3$	Horse Manure + $CaCO_3$
May, 1914	.08	.20	.02	.09	.43	.30	.06	—.01	.06	.06
Nov. 25–Feb. 16	.12	.09	.30	.03	.01	.13	.09	.03	.12	—.04
Feb. 16–Apr. 13	.04	—.06	.02	.02	.01	.04	.06	.10	—.01	.10
Apr. 13–June 1	.05	—.06	—.10	—.10	.03	.01	.02	—.03	.57	.04
June 1–Oct. 15	—.07	.....	—.09	—.09	—.05	—.06	.18	.23	—.30	.05
Gm. of Corn Stalks Produced (wt. when cut)										
Yield 1915	601.9	443	1174	165.9	928	620.7	123	46.6	637	386
Yield 1916	160.0	347	392	308.0	249	260.0	222	271.0	401	456

the other was never sterilized. The amount of 1 per cent acid and 4 per cent NH_4OH-soluble matter was determined in December, 1916. Leaves which had fallen in September, 1916, also were studied by determining

TABLE V

PER CENT OF HUMUS IN GREEN MANURES

	Oct. 1915	Dec. 1915	Feb. 1916	Mar. 1916
Green cowpea stalks mixed with soil—				
Limed	....	0.75	0.75	0.65
Unlimed	0.63	0.84	0.83	0.66
Green alfalfa—				
Limed	....	0.64	0.66	0.77
Unlimed	0.67	0.57	0.62	0.65
Green sweet clover—				
Limed	....	0.77	0.78	0.71
Unlimed	0.40	0.84	0.62	0.58
Green oat stalks—				
Limed	....	0.87	0.65	0.80
Unlimed	0.46	1.09	0.92	0.81
Check	0.40	0.42	0.43	....

TABLE VI

GAIN OR LOSS IN PERCETAGE OF HUMUS

	Cowpeas	Alfalfa	Sweet Clover	Oats
Check	0.21	0.25	—0.02	0.04
NH_4OH—soluble when mixed				
Oct. 15–Dec. 15	0.21	0.10	0.37	0.41
Dec. 15–Feb. 16	—0.01	0.05	0.01	—0.22
Feb. 16–Mar. 16	—0.07	0.03	—0.07	0.15

TABLE VII

YIELD OF CORN IN TEST WITH GREEN MANURES

	Height 6-27-16 inches	Weight of Corn and Stalk (when cut) gm.
Cowpea—		
Disced	16.5	403
Rolled under	26.5	487
Dried	22.5	300
Limed	24.0	497
Average	22.4	421.8
Alfalfa—		
Disced	19.5	368
Rolled under	21.5	499
Dried	27.5	435
Limed	23.5	518
Average	23.0	455
Sweet Clover—		
Disced	21.0	297
Rolled under	21.5	413
Dried	26.5	400
Limed	24.0	550
Average	23.0	415
Oats—		
Disced	29.0	518
Rolled under	31.5	485
Dried	29.5	292
Limed	28.0	533
Average	29.5	457

the solubility in acid and alkali. Some of the leaves were kept dry while other were placed in piles under boards out-of-doors and allowed to decompose until March, 1917. Table VIII gives the solubility changes.

TABLE VIII

SOLUBILITY OF MAPLE LEAVES IN ACID AND ALKALI

Sterilized leaves, 3 years old....	HCl-soluble volatile matter.....	27.60%
	NH_4OH-soluble volatile matter.....	11.22%
Unsterilized leaves, 3 years old..	HCl-soluble volatile matter.....	26.91%
	NH_4OH-soluble volatile matter.....	17.82%
Dry leaves, Sept. 16–Mar. 17...	HCl-soluble volatile matter.....	9.23%
	NH_4OH-soluble volatile matter.....	20.78%
Rotted leaves, Sept. 16–Mar. 17.	HCl-soluble volatile matter.....	7.59%
	NH_4OH-soluble volatile matter.....	26.31%

Discussion of Results

The rate of breaking down of many of our common manures differs so that the plant-food contained becomes available to the plant at varying intervals. Table IV shows that fresh cow manure and steer manure are very soluble in NH_4OH, whereas horse manure is quite insoluble in it. This insolubility is evidently related closely to the release of plant-food, because, as shown in figure 3 and Table IV, there was no appreciable growth of corn produced in the horse manure boxes, except in Box 9 which was affected by other conditions. The humus showed an increase in these boxes after it was too late for the first crop, but it was more available for the second, as the corn yield indicated. In the case of Box 9, dolomitic limestone seemed to hasten the decomposition of the manure, resulting in a higher corn yield the first year and a lower yield the second. Among the green manure boxes, the oats humified rapidly. This indicated a greater corn yield than in the other green manures and the large harvest verified it. Table VII seems to show that the rolling under of green manures causes a more rapid decomposition than either the discing or the plowing under of the green mass after it has dried. Wherever there was a large amount of NH_4OH-soluble matter, either without humifying or where humification takes place rapidly, as in cases of cow, steer and green oat manures, the growing plant got an early start and produced good growth. Just the opposite seems to be true of horse manure, hence it appears that it would be the least satisfactory of the common manures to use on lawns and in greenhouses where quick action is desired. Much of the humification work mentioned in the introduction was conducted with substances other than farm manures and in greenhouses and cellars. It is almost impossible to compare these results with those brought about under outside weathering agencies.

Conclusions

1. The results of the vegetation and humification tests would seem to show that whenever there is rapid humification of manure, the growth of the plant is greatly stimulated, indicating that "the decay of organic matter is desirable in plant growth and not just its mere presence." This was especially noticeable when green manures were rolled under and limed as compared with discing or mixing the manures uniformly with the soil.

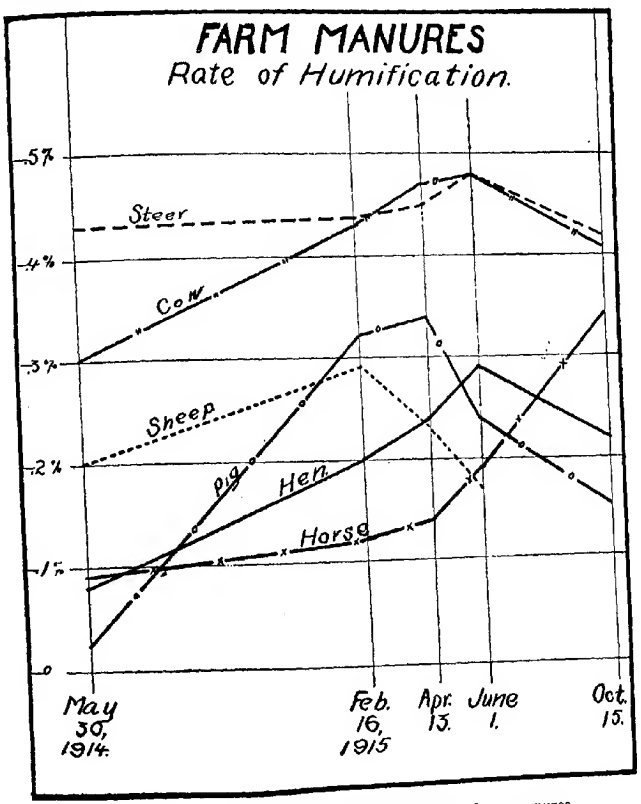

Fig. 1.—Curves showing rate of humification in farm manures.

2. Certain of the manures seem to be as soluble in a 4 per cent ammonia when just mixed with the soil as after humification. This was found to be true with alfalfa and steer and somewhat with cow manures (fig. 1 and 2).

3. Horse manure seems to humify slowly and its plant-food was largely unavailable to corn during the first year, but the humification and vegetation tests show it becomes more available in the second year. It

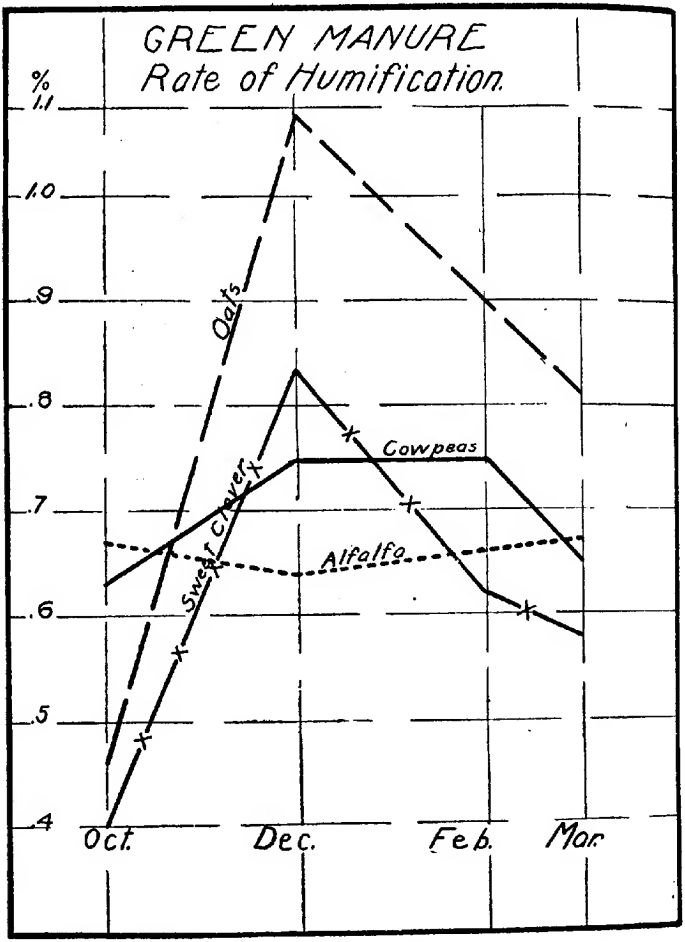

Fig. 2.—Curves showing rate of humification in green manures.

was possible to increase the rate of humification of horse manure in the first year by adding dolomitic limestone, which resulted in a greater yield of corn than where humification had not taken place (Table IV and fig. 3).

4. The organic residues left in the soil from manure treatment was not very effective during the second year in producing growth of corn, probably because the most available or valuable complexes had disappeared in the first year.

5. There is no apparent relationship between the percentage of ash in humus and the growth of corn.

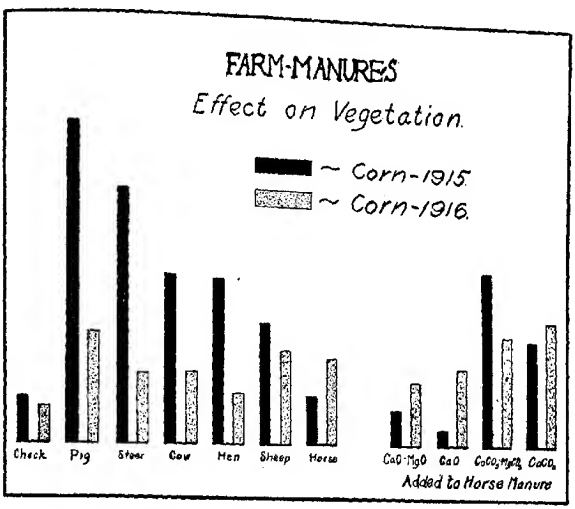

Fig. 3.—Diagram showing effect of farm manures on corn, 1915 and 1916.

6. The humification and vegetation tests would seem to indicate a rather close relationship between the amount of humus and the growth of corn, as shown in the graphs.

Literature Cited

(1) Brigham, R. O.
1917. Assimilation of organic nitrogen by *Zea Mays* and the influence of *Bacillus subtilus* on such assimilation. *In* Soil Sci., v. 3, p. 155-191.

(2) Fraps, G. S., and Hammer, N. C.
1910. Studies of the ammonia-soluble organic matter of the soil. *In* Tex. Agr. Exp. Sta. Bul. 129, Pt. 2, p. 23-30.

(3) Gortner, R. A.
1916. The organic matter of the soil: III. On the production of humus from manures. *In* Soil Sci., v. 3, p. 1-8.

(4) Grandeau, Louis.
1872. Richerches sur le role des matieres organiques du sol dans les phenomenes de la nutrition des vegetaux. *In* Compt. Rend. Acad. Sci. (Paris), t. 4, p. 988-991.

(5) POTTER, R. S., and SNYDER, R. S.
1915. Ammo-acid nitrogen of soil and the chemical groups of amino acids in the hydrolyzed soil and their humic acids. *In* Jour. Amer. Chem. Soc., v. 37, no. 9, p. 2225.
(6) SMITH, O. C.
1915. Humus determinations. *In* Jour. Assoc. Off. Agr. Chem., v. 1, p. 46.
(7) WEIR, W.
1915. The effect of removing the soluble humus from a soil on its productiveness. *In* Jour. Agr. Sci., v. 7, p. 253.

THE EFFECT OF STERILIZATION OF SOILS BY HEAT AND ANTISEPTICS UPON THE CONCENTRATION OF THE SOIL SOLUTION[1]

By

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Sterilization of soils is an old practice. Centuries ago, before microbes were discovered, the Romans carried on the practice of "firing" the surface of the soil. They well appreciated increase in crops resulting from this crude method.

The use of antiseptics as a means of killing soil pests dates back to 1877, when Oberlin (6) used carbon bisulfide to kill *phylloxera*. Ten years later, Girard (4) used this antiseptic to destroy a nematode-infested soil. Dating from these periods until the present, various treatments by means of heat, as well as with many kinds of antiseptics, have been carried out for the purpose of controlling soil pests.

Until 1888 bacteriologists thought that sterilizing the soil merely destroyed the living forms therein. At this time, however, Frank demonstrated that the soluble materials in the soils are greatly increased by such treatments. This was further substantiated by Pfeiffer and Franke (7) in 1896, and by many others. Recent literature on this point, by Pickering (8), Lyon and Bizzell (5), Russell and Hutchinson (9), Schreiner and Lathrop (10), Seaver and Clark (11), and Coleman, Lint and Kopeloff (2), all indicate that heating the soil or applying antiseptics increases its water-soluble extract.

Does sterilization by ordinary methods in the greenhouse or in the laboratory increase the concentration of the soil solution, and if so, to what extent? In a recent publication, Bouyoucos (1) showed that by autoclaving soils with high moisture contents for a period of 3 hours, the concentration, as determined by the depression of the freezing point, was appreciably increased.

To collect further data on this particular subject the writer carried out the following experiments:

1. To note the effect of sterilization by commercial methods, as practiced in the greenhouse, upon the concentration of the soil solution.
2. To determine the effect upon concentration of sterilization methods as adopted in biological laboratories.
3. To demonstrate what effect the presence of organic matter has upon concentration in the process of sterilization.

[1] Received for publication January 26, 1917.

Commercial Methods of Sterilization[2]

Several soils were sterilized by methods (steaming and applying formalin) as nearly as possible approaching those used commercially.

Sterilization with Steam. In sterilizing by steam about 30 pounds of the moist soil, in a large jar, was placed in the autoclave and the pressure of the steam maintained at 10 pounds for 1 hour.

Sterilization with Formalin. The method of sterilizing by means of formalin (1-50) consisted of the usual procedure of adding 6 liters of the solution containing 1 part of commercial formalin to 49 parts of water, to 30 pounds of soil held in shallow wooden boxes which were not water-tight. This was allowed to stand, with stirring at regular intervals, for 2 weeks, at the end of which period the samples were drawn. In the case of the other formalin treatment, 6 liters of a solution composed of 1 part of formalin to 99 parts of water were added to 30 pounds of soil.

Sterilization with Steam and Formalin. One series of soils received the formalin (1-50) treatment as above, and after standing for a period of 24 hours was sterilized in the autoclave at 10 pounds for 1 hour. The other series of the same soils was first sterilized in the autoclave at 10 pounds for 1 hour and then received the formalin (1-100) treatment.

Manipulation in Determining Concentration

Two weeks after the soils had been sterilized they were brought to the laboratory and tested for concentration. After all the samples had been air-dried and sieved through a 20-mesh sieve, they were subjected to analysis. To a 20-gm. portion of each sample of soil enough distilled water was added so that a desirable consistency of soil plus water resulted when mixed by hand. After thorough mixing, these samples were placed in large hard-glass test tubes, which were then tightly stoppered and allowed to stand for a period of 12 to 16 hours, or until the moisture was evenly distributed throughout the soil. The concentration of the soil solution was determined by means of the Beckmann thermometer as has been recommended by Bouyoucos (1).

By glancing through Table I it is apparent that sterilization by the so-called "commercial" method increases the concentration of the soil solution in practically all instances. It is seen that this increase is dependent upon the character of the soil as well as the method which is adopted for sterilization.

Applying heat alone is quite effective in increasing the soil concentration, in that as much as 0.56 atmosphere increase is noted in the loam soil steamed over the loam untreated. With the sandy soil, however, heating increased the concentration only one-fourth as much as that of the loam soil.

[2] The writer is indebted to Mr. Monteith, who sterilized these soils for his work on the "damping-off" fungi and the effect of soil sterilization upon seedlings. It was through his kindness that the writer was permitted to take samples of these soils sterilized by the different methods.

Sterilization by means of formalin did not seem to increase the concentration as much as heating. In the heavier soil the greatest increase was apparent in the 1-50 treatment; however, the difference is not great. Upon inspecting the data showing the effect of formalin treatments upon samples of sandy soil, one notes a depression in concentration over that of the check. This is readily explained by the fact that, because of the application of more solution than the soil could hold against gravity, a considerable amount of the material already soluble and more that was made soluble in the soil was leached away. Hence the importance of not flooding and leaching is apparent, for this would become a very potent factor in greenhouse practices, especially when heavy applications of readily soluble fertilizers are used.

TABLE I

THE EFFECT OF STERILIZATION BY STEAM AND FORMALIN UPON THE CONCENTRATION OF THE SOIL SOLUTION

Kind of Soil and Treatment	Depression of Freezing Point: °C. Average[1]	Concentration: Osmotic Pressure Atmospheres	Increase in Concentration due to Treatment Atmospheres
Loam			
Untreated	.044	0.530	.....
Steamed	.091	1.097	0.567
Formalin (1-50)	.070	0.844	0.314
Formalin (1-100)	.065	0.784	0.254
Formalin (1-50) and steamed...	.135	1.563	1.033
Steamed and formalin (1-100)..	.062	0.748	0.218
Sandy			
Untreated	.080	0.965	.....
Steamed	.092	1.104	0.144
Formalin (1-50)	.053	0.639	—0.326
Formalin (1-100)	.068	0.820	—0.145
Formalin (1-50) and steamed...	.104	1.254	0.289
Steamed and formalin (1-100)..	.092	1.109	0.144

[1] At least three and sometimes more determinations were made with each treatment and these figures represent the average of the several determinations.

The error in experimentation was less than 5 per cent.

The greatest effect of sterilizing agents upon soil concentration is appreciated in the sample of loam soil which was first treated with formalin and then steamed, in which case the concentration was increased from 0.53 to 1.56 atmospheres. The effect of leaching is again apparent in the loam soil steamed and then treated with formalin (1-100). The concentration of the sandy soil was increased 0.289 atmosphere where formalin (1-50) was applied and then steamed. Comparing this treatment with the formalin (1-50) treatment shows that even though much had been leached away in the first treatment, the constituents of the soil were enough decomposed so that steaming was more effective in increasing the concentration than was realized when this soil was only steamed. Steaming and then applying formalin (1-100) to the sandy soil was only one-half as effective as where formalin (1-50) was applied and then steamed.

Laboratory Methods of Sterilization Affecting Concentration

One hundred-gm. quantities of three typical soils (Sassafras loam, Penn loam, and Norfolk sand), after having been air-dried and sieved through a 20-mesh sieve, were weighed out into 250-c.c. Erlenmeyer flasks. Enough distilled water was added to bring the moisture content up to the physical optimum, or 40 per cent of the water-holding capacity, as determined by the funnel method. These soils were then sterilized in the autoclave at 15 pounds pressure for 15 minutes, after which they were again air-dried. Blank samples of the same soil received the same treatment, with the exception of sterilization, thus eliminating the possible discrepancy which might arise as a result of insoluble material being made available by wetting and drying. Twenty-gm. samples of the three soils were then weighed out, water added to bring the moisture to the physical optimum, and then the concentration determined in the same manner as in the previous cases.

TABLE II

THE EFFECT OF STERILIZATION BY STEAM AS APPLIED IN THE LABORATORY UPON THE CONCENTRATION OF THE SOIL SOLUTION

Kind of Soil and Treatment	Depression of Freezing Point °C.	Concentration: Osmotic Pressure Atmospheres	Increase in Concentration due to Treatment Atmospheres
Sassafras loam not sterilized..	0.042	0.506	.....
Sassafras loam sterilized.....	0.062	0.749	0.243
Penn loam not sterilized.....	0.610	0.734	.....
Penn loam sterilized.....	0.860	1.037	0.303
Norfolk sand not sterilized....	0.980	1.181	.....
Norfolk sand sterilized.....	0.970	1.170	0.000

Steaming the soil for 15 minutes at 15 pounds affected the concentration of two of the soils, as shown in Table II. The concentration of the Sassafras soil was increased one-fourth of an atmosphere, while that of the Penn loam was slightly greater. No increase in concentration, however, could be detected in the determinations with the Norfolk sand. This points to the fact suggested by Bouyoucos (1), that "the magnitude of the increase is by far greater in those soils in which the organic matter is very high or predominates, than in mineral soils."

Effect of Organic Matter Upon the Concentration of the Soil Solution During Sterilization

Inasmuch as the concentration of the soil solution in the Norfolk sand was not increased by steaming in the autoclave at 15 pounds for 15 minutes, as noted above, the writer next tried to find out what effect the presence of ordinary organic matter, as used in biological work, had upon concentration when the soil plus organic matter was subjected to sterilization by steam. Consequently, 1 per cent of dried blood and 2

per cent of cottonseed meal were mixed, respectively, in 100-gm. quantities of Norfolk sand, the moisture added to physical optimum, the soil sterilized, and the concentration determined as before.

The presence of organic matter was effective in increasing the concentration of the soil solution when steamed. One per cent of dried blood had little effect, however, in that an increase of only 0.09 atmosphere was realized, while 2 per cent cottonseed meal was more effective in increasing the concentration, in which case 0.24 atmosphere was found to be the difference between the respective concentrations of the sterilized and the unsterilized samples. This points to the fact that the

TABLE III

THE EFFECT OF STERILIZATION BY STEAM AS APPLIED IN THE LABORATORY UPON THE CONCETRATION OF SOIL RECEIVING APPLICATIONS OF ORGANIC MATTER

Treatment	Depression of Freezing Point °C.	Concentration: Osmotic Pressure Atmospheres	Increase in Concentration due to Treatment Atmospheres
1% Dried Blood not sterilized.....	.124	1.451	.....
1% Dried Blood sterilized.....	.134	1.559	0.090
2% Cottonseed Meal not sterilized..	.248	2.990	.....
2% Cottonseed Meal sterilized.....	.268	3.231	0.241

extent to which the presence of organic matter would increase soil concentration caused by sterilization would be dependent upon the kind, and no doubt the amount, of the organic matter present.

SUMMARY

The following conclusions may be drawn as the result of the preceding study:

1. The lowering of the freezing-point method is a satisfactory means of determining soil solution concentration as influenced by sterilization.

2. In commercial as well as laboratory methods of steaming soils, the heavier soils are more influenced by sterilization than lighter soils.

3. Steaming alone was more effective in increasing the concentration than either of the formalin treatments.

4. Applying formalin (1-50) and then steaming at 10 pounds pressure increased the concentration more than any other method tried. By this method the concentration was increased to 3 times the original concentration of the soil solution.

5. A considerable amount of soluble material is leached out of the soil, and thus the concentration is lowered if the quantity of antiseptic solution applied is so great that the soil cannot hold it against the force of gravity.

6. The Sassafras loam and the Penn loam soils were affected in the laboratory sterilization method so that the concentration was increased 0.24 and 0.30 atmosphere, respectively.

7. The concentration of the Norfolk sand containing a very small amount of organic matter was not affected so that it could be detected by the method employed.

8. One per cent of dried blood increased the concentration of the soil solution of the Norfolk sand 0.09 atmosphere, while 2 per cent cottonseed meal increased the concentration three times this amount.

Literature Cited

(1) Bouyoucos, George J.
1915. The freezing point method as a new means of measuring the concentration of the soil solution directly in the soil. Mich. Agr. Exp. Sta. Tech. Bul. 24, 44 p.

(2) Coleman, D. A., Lint, H. C., and Kopeloff, N.
1916. Can soil be sterilized without radical alteration? *In* Soil Sci., v. 1, no. 3, p. 259-274.

(3) Frank, B.
1888. Ueber den Einfluss welchen das Sterilisiren des Erdbodens auf die Pflanzen-Entwickelung ausubt. *In* Ber. Deut. Bot. Gesell., Bd. 6, p. 87-98.

(4) Girard, Aime.
1894. Recherches sur l'augmentation des recoltes par l'injection dans le sol du sulfure de carbone á doses massives. *In* Bul. Soc. Nat. Agr. France, p. 356.

(5) Lyon, T. L., and Bizzell, J. A.
1910. Effect of steam sterilization on the water-soluble matter in soils. *In* N. Y. (Cornell) Agr. Exp. Sta. Bul. 275.

(6) Oberlin, C.
1894. Bodenmüdigkeit und Schwefelkohlenstoff. Mainz.

(7) Pfeiffer, Th., and Franke, E.
1896. Beitrag zur Frage der Verwertung elementaren Stickstoffs durch den Senf. *In* Landw. Vers. Stat., Bd. 46, p. 117.

(8) Pickering, S. U.
1908. The action of heat and antiseptics on soils. *In* Jour. Agr. Sci., v. 3, p. 32-54.

(9) Russell, E. H., and Hutchinson, H. B.
1909. The effect of partial sterilization on the production of plant-food. *In* Jour. Agr. Sci., v. 3, p. 111-144.

(10) Schreiner, O., and Lathrop, E. C.
1912. The chemistry of steam-heated soils. U. S. Dept. Agr. Bur. Soils, Bul. 89.

(11) Seaver, F. J., and Clark, E. D.
1912. Biochemical studies on soils subjected to dry heat. In Biochem. Bul., v. 1, p. 413.

THE SOIL SOLUTION OBTAINED BY THE OIL PRESSURE METHOD[1]

By

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Introduction

The study of the soil, the medium in which the plant grows, has been approached from many angles. The sum total of these efforts has thrown some light on what the soil is, what it lacks for good plant growth, and thus what it requires to make up this deficiency. The one phase which has been studied to the least extent is that of the actual medium from which the plant takes part of its nourishment, that is, the soil solution.

Soil solution is a homogeneous mixture of the water (the solvent) and the soluble ingredients of the soil (the solutes), consisting of gases, liquids and solids. These solutes may influence the solubility of other substances.

The maximum amount of the soil solution that the soil can hold depends upon the physical character of the soil—the finer a soil and also the more organic matter, humus, present, the more the soil will hold on account of the larger surface for adsorption. The capillary water present is more firmly held by the force of surface tension.

In order that the plant may take up through its roots the mineral constituents in the soil, the latter must be in solution. As the plant requires moisture for its existence, the soil water performs a double function—it furnishes the moisture to the plant, and holds in solution the mineral and the organic compounds. The solutes that are present assist by dissolving some of the constituents of the soil otherwise insoluble. The character or type of soil, *e. g.*, the size and composition of soil particles, will determine to a large extent what the soil solution contains. Treatment of the soil in regard to cultivation, drainage, fertilizer and cropping has a marked influence upon the amount and kind of solutes present.

Gedroitz (7) says that the greatest part of the soil compounds has a very low degree of solubility. The solubility of the soil compounds is not a constant quantity, but depends upon temperature and pressure and, as stated previously, upon the solutes present. Practically every soil contains all the common rock-forming minerals (4; 5, p. 9), all of which

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are more or less soluble in water, depending somewhat, as to the amount dissolved, upon such factors as temperature, pressure, etc. Not only these mineral compounds, but also the organic compounds formed from decaying plant and animal tissues are found in solution.

A very important factor was brought out by Gola (8) in regard to the colloidal character of the soil particles and its effect upon the soil solution. On account of the complexity and colloidal nature of some of the soil particles, it is claimed that the colloids adsorb some of the salts of the solution. The amount adsorbed depends upon the moisture content. Presence of a large amount of water sets up a dialysis which causes the hydrogels to give up the salts adsorbed. Reverse action takes place at first with a diminution of the moisture content, but finally the imbibition water of the hydrogels evaporates and the gel is precipitated as an amorphorous mass, the bonds uniting the salts with the collolidal molecule are broken and the water set free is able to dissolve more salts than it can before the loss of the adsorbent properties of the soil.

The Real Importance of the Soil Solution

The study of the soil solution is of great importance from various points of view. The very complexity of the soil and the constant change which the soil is undergoing introduce a countless play of factors. This complexity inherent in the subject should not discourage investigation. It must be admitted that the soil solutions from different fields or from different parts of the same field will not be exactly the same, nor even will the soil solutions present at any place remain constant. But the same could be urged against chemical analysis of the soil which likewise varies, yet which on the whole gives a general idea of the field conditions.

The soil solution, at the outset, may be considered in its relation to soil formation. Zacharov (18) maintains that the soil solution plays an essential role to the extent in which it relates to the process of leaching, dissolving and further advancement of the soil particles in the soil layer. Finding that the soil extract from different soils varied in color, reaction and amount and composition of the soluble constituents, he was able to differentiate the different soil types. The amount of humus present has a marked influence upon the color and reaction of the soil solutions. Soils of the same composition as regards type but differing in organic matter present will vary in their soil solutions as to color and reaction. Not only the humus, but also certain of the mineral constituents of the soil have a marked influence upon the color of the soil solution.

The second phase of importance from an ecological standpoint is the soil solution as the medium for plant growth—the actual medium in contact with the plant roots, and the substratum for the microbial life. The plant is susceptible to the reaction whether it is neutral, alkaline, or so-

called acid and to the amount and kind of available substances present, especially of potassium, phosphorous, calcium and nitrogen in their various forms.

Cameron and Bell (5, p. 8) say, "The soil solution is physiologically of the greatest importance, as it is the source from which plants absorb the mineral constituents which have been demonstrated to be absolutely essential to their continued existence and development. The study of the soil solution, therefore, becomes of the first importance in the investigations of the relation of the soil to plant growth."

Heretofore no available means have been devised for obtaining large amounts of the true soil solution or a fair representative of the same. From the study of synthetic solutions and of water extracts, much light has been thrown upon soil fertility. With a true soil solution, much more valuable information ought to be obtained. For example, we have used the soil solution obtained by the method herein described to study the effect of the microorganisms in fertilized soils and also to show the effect of soil reaction upon the number and kind of microorganisms present. This phase of the work will be taken up in a later publication.

Previous Methods

Various investigators have tried to find some means of obtaining a soil solution as it exists in the soil in order that they may become better acquainted with the medium in contact with the plant roots and the medium in which microbial activities must take place. Among the first tried may be mentioned the drainage waters (9, p. 22-25; 11).

1. *Drainage Waters*

Some pertinent objections may be brought against this method. The gravitational or drainage water as it moves downward through the soil carries with it only a small part of the film water, but if the flow is continued long enough this film will be entirely replaced by a new one which for the time being is not concentrated to the same degree as the one before it. Schloesing (14, p. 99) claims that drainage water collected after it has passed through a meter of earth does not faithfully represent the solution which the arable layers imbibe. In all drainage water, according to Hilgard (9, p. 271) the chief nutritive ingredients of plants except nitrogen are present in traces only. The drainage waters, therefore, contain too much of some ingredients and too little of others to give any definite information as to the soil requirements.

2. *Soil Extracts*

Soil extracts have, no doubt, been used more than any other method for the study of the soil. It depends upon whether a total analysis of the soil is desired, or whether just the ingredients that may become easily

available to the plant are desired, as to the nature and strength of solvent used. Some investigators use mineral acids, others organic acids and still others use water.

In all such extractions, none deals with a solution which contains the exact amount of substances available to the plant. It is seriously questioned if they give data on actual soil conditions. Consequently water, the least objectionable, has been used as a solvent for determining the available plant nutrients in the soil. While Briggs' method of separating the turbid supernatant liquid by forcing it through a Pasteur-Chamberland filter or the other common method of separation by filtering through filter paper, is obviously more applicable for yielding data than extractions with acids and the like, yet it yields only dilute washings of the soils which cannot be concentrated to reproduce the original solution.

3. *Artificial Root*

In their study of capillary movement of the soil moisture, Briggs and McCall (2) tried to imitate the plant root in its adsorption of moisture by using a close-grained porcelain filter connected to an exhausted receiver and placed in the soil. It is claimed that the solution thus obtained is identical in concentration and composition with the soil solution from which plants get their food. The disadvantage is that it is applicable only to those soils of comparatively high moisture content and that it furnishes only a small amount of solution. The adsorption by the clay filter itself is known to be important enough to alter seriously the solution obtained.

4. *Centrifuge*

Briggs and McLane (15) tried to obtain the soil solution by means of whirling the moist soil at a very high speed. Thus they were able to reduce the soil moisture to approximately the optimum condition for plant growth and this method therefore is limited to soils containing more than the optimum. Nevertheless by this method it has been possible to gain some valuable information concerning the actual concentration of the free soil moisture. For any extensive study the amount of solution obtained is comparatively so small as to be practically prohibitive.

5. *Displacement Methods*

Among the first attempts to obtain the soil solution in an unaltered condition was that of Th. Schloesing (13; 14, p. 98) in the '60's. In this method pure water was carefully added so as to force out the water already in the soil. To make the line of demarcation between the added water and that already in the soil he colored the water with carmine. The objection raised to this method by Itscherekov (10, p. 147) was that it did not work well with soils containing less than 20 per cent of moist-

ure and the time required was too long. Itscherekov conceived the idea that the attractive forces of the soil particles would be more easily overcome and also that the capillarity would not be destroyed, but somewhat modified, by using a liquid lighter than water that would wet the soil and penetrate the smallest pores. Otherwise, the soil solution would rise above the displacing liquid and lessen the mechanical mixing of the two substances. For this purpose he (10) selected alcohol, either methyl or ethyl. It is claimed that soils with a moisture content of 10 to 2 per cent were extracted with success. The chief objection is that alcohol mixes to some extent with the soil solution, and also, that the alcohol is an active agent dissolving and precipitating out certain of soil ingredients.

Gola (9, p. 22-25; 16) tried an imitation rain and pressure method. After allowing an excess of water to drain off, he subjected the soil to the pressure of a screw press. The chief objection that Stiles and Jorgensen (16) raise to this method is that it is not applicable to all kinds of soils, but it may be used for all soils having a high water capacity. Hesselhink van Suchtelen (17) impressed by the feasibility of the Itscherekov's method modified the plan and after some preliminary work selected a thin paraffin oil, later a thicker and heavier one as a displacing liquid for obtaining the soil solution in its natural condition. The oil was drawn through the soil placed on a large Büchner funnel by means of suction. The pressure thus obtained was too low for satisfactory results.

Present Method

Paraffin Oil Displacement—Pressure Method

Later, instead of using suction as described above, a pressure method was undertaken by van Suchtelen and Itano with the idea that if a greater force could be applied than could be obtained with the suction pump, better results would be attained. Therefore, another apparatus was necessary. In this apparatus the displacing liquid was forced through the soil instead of being pulled through by suction. It is with this apparatus and with the solution obtained that the author has been experimenting for over two years.

Apparatus

This consists of a cylinder connected at the top with a high-pressure pump and open at the bottom with a small-bored spout from which the soil solution flows. The details of the construction may be seen in Plate I. After trial with a cast-iron cylinder 5 inches in diameter and 22 inches long, others were made later of larger dimensions, 6 by 24 inches. The cylinder is screwed into a flange and reducer. The union is soldered in order to make a more perfect joint. The reducer is connect-

ed to the pedestal, the upper part of which is hollow, while the part below the spout is plugged (to prevent loss of solution). This entire lower part can be easily removed from the cylinder for cleaning. The cap is fastened on with bolts. A gasket of copper and asbestos is used to make a tighter joint. In the bottom of the cylinder is placed a perforated plate and above this two fine wire gauzes to hold back the soil. These gauzes are larger than the plate to prevent the soil from passing under its edge. A series of cylinders can be arranged in a battery, so that all can be worked at the same time, or any one or two cut out. In our experiment, three were so connected. For this purpose any kind of hand pump can be used that will force the liquid through under pressure. The gauge is an ordinary 500-pound steam pressure gauge and is placed on the main line so that the pressure can be read on any cylinder of the battery, by putting in or cutting out the cylinders from the pressure pump.

The small cylinder (5 by 22 inches) is capable of holding 18 to 30 pounds of soil, depending upon the nature of the soil, while the larger ones (6 by 24 inches) hold 35 to 50 pounds.

Before using the cylinders the inside is coated with hot paraffin; in order to keep the soil and the solution away from the iron. The lower pipe and spout, as well as the plate and gauze in the bottom, are treated in a like manner.

The cylinder is weighed before and after filling in order to get the weight of the soil used. The soil used is thoroughly mixed by rolling it back and forth on a large piece of oil cloth. The soil is added in small quantities (about a double handful) at a time, a small portion being saved each time for a composite moisture sample. The soil in the cylinder is thoroughly packed down by means of a stick with an iron disk on the end. A great deal depends upon the packing. If loosely packed, or unevenly packed, there will be left an easy channel for the displacing liquid to pass without much pressure being exerted and also without obtaining the total amount of solution possible. The pressure exerted will depend upon the moisture content of the soil. It is raised gradually as long as moisture continues to come until finally 500 pounds pressure is reached if the oil has not come through before the pressure reaches this maximum. One reason why the pressure is raised gradually is to give the soil an opportunity to adjust itself and thus to prevent the oil from passing through too soon as a result of the formation of channels. Some soils with a very high moisture content, even on packing, will give off some of the solution that they contain.

When the oil comes through in any of the cylinders, the valves on the others are closed to hold the pressure attained, while the pressure is being released on the first by opening the release valve on the pump. After

this, the action is reversed and the pumping is continued as before. When all the cylinders are used for extraction of the same soil, all extractions, if mixed, will give an abundance of material to work with. In the sandy soils oil will makes its appearance generally from 3 to 9 hours, while with the finer-grained soils the oil may not come through for several days. Solutions for analysis are hardly ever used after a protracted (48-hour) extraction. Solutions are gathered from time to time and placed in a refrigerator to retard the bacterial action. Care is taken to prevent chemical changes in the solution by using resistance glass.

When cleaning out the cylinders, the oil that is superimposed on the soil is saved and can be used again provided one strains it to get rid of the dirt that it might contain. It is found that in order to clean out the cylinders easily, it is best to bore into the soil either with an ordinary soil auger or with an ordinary 3-inch bit (with the former several holes will be necessary), then with a bar the soil may be pried from the sides and taken out quite readily. With heavy soils like clay, chunks of soil may be pulled out with the soil auger which acts as a cork-screw. To remove the soil in the bottom of the cylinder unscrew the cylinder from its pedestal and invert it, then, through the opening in the bottom, one can force the soil out by driving on the perforated plate with a piece of pipe and hammer. The soil in the bottom will be found to be packed harder and drier than that at the top.

The inertness of paraffin oil gives it an advantage over alcohol; it does not act upon the soil nor does it mix with the soil solution. Whatever oil comes through with the solution can easily be separated by cooling the flask so that the oil will adhere to the sides, and then pouring off the water or by using a separatory funnel.

The method can be used with all kinds of soils, but it is better adapted to the coarser and more porous soils like the sands and sandy loams than to the finer-grained and less porous soils, such as the clays and clay loams. In nearly all of the extractions thus made, a thick, viscous filtered oil of cylinder stock was used. If the oil at times became so thick as to prevent its entering the pump, it was thinned with a thin paraffin oil.

Moisture Content of Soils and Solution Obtained

This method will not give the entire moisture content, nor will any method yet devised give the entire moisture content as it exists in the soil. The moisture content that is necessary for an extraction depends upon the nature of the soil used. The greater the amount of moisture present up to its total water capacity, the larger is the amount of solution that can be procured. It is assumed that, everything else being equal, all the solution above a certain point can be obtained. If we consider the work of Briggs and McLane (3) we note the force with which the water

film is held to the soil particles. In the whirling of moist soils, they found that this film was not removed by a force of 3,000 times the force of gravitation. In the paraffin oil pressure method, the highest pressure used was only 550 to 600 pounds, or about 40 atmospheres. The thicker the film around the soil particles, the smaller is the amount of force required to remove the moisture from the outer edge of the film. Lord Rayleigh (12), as a result of some experiments, calculated that the thin film was held with a force as high as 25,000 atmospheres. With part of the moisture held by that great force, it would be impossible to get it all with 40 atmospheres.

Since the oil penetrates some soils only in part and other soils entirely, the amount of moisture that remains is calculated by the difference between the total moisture content of the soil and that obtained by extraction.

Properties of Soil Solution

The study of certain of the physical, chemical and biological properties of the soil solution ought to give some information in respect to the soil problems.

TABLE I

MOISTURE CONTENT OF THE SOIL AND AMOUNT EXTRACTED

Soil	Per cent of Moisture in Soil before Extraction[1]	Number of Soils	Number of Determinations	Per cent of the Total Moisture in Soil Extracted[2]
1. Fine sand (surface)	42.02— 46.73	5	10	11.10—29.14
2. Fine sandy (surface)	7.66	1	2	35.17
3. Fine sandy (surface)	29.74	1	2	30.48
4. Fine sandy (subsoil)	17.35— 23.18	4	8	42.88—74.08
5. Medium sandy loam (surface)	27.24— 29.36	2	6	11.19—17.92
6. Medium sandy loam (surface)	45.38	1	2	27.25
7. Clyde F. S. loam (surface)...	40.57— 43.82	3	4	1.50— 8.50
8. Miami silt loam (surface)....	35.12— 37.81	3	6	4.60—22.80
9. Clyde clay loam (subsoil)....	40.86	1	2	5.18
10. Miami clay (surface)	24.52	1	1	5.80
11. Peat (surface)	132.90—251.95	2	2	14.60—23.40
12. Muck (surface)	98.22—164.00	3	5	2.00—12.68

[1] Based on oven-dried soil. [2] Based on total moisture in soil.

One of the important factors in the study of the soil solution is its concentration because of the bearing it has upon the development of the living cell. This form of concentration concerns not only the amount of total solids present, but still more the effect it has on the depression of the freezing point. The latter determines the force or pressure that the solutes exert when placed under varying conditions. The specific gravity, surface tension and viscosity are very important, also, in that they have much to do with the capillary movement of the soil water. The greater the surface tension, the thicker will be the capillary film and the

harder it will be to remove this film. The more viscous the liquid is, the less will be the movement due to capillary action. The reaction is worthy of consideration both in case of the microorganism and of the plants, for upon this depends the development and growth of the lower and the higher forms of life.

The results in Table I are based on duplicate determinations except No. 10, two determinations of No. 8, and one of No. 12. Where more than one soil is used the range of the moisture content of those soils and the range of percentage of total moisture extracted are given. In all other cases the results are based on average determinations.

TABLE II

TOTAL SOLIDS OF SOIL SOLUTIONS FROM SOILS EXTRACTED

Soils								
1. Fine sandy (surface)	M.C.	7.66	29.74	40.77	42.02	43.98	46.02	46.73
	T.S.	400	700	1960	1809	1675	1380	1820
2. Fine sandy (subsoil)	M.C.	17.35	17.71	21.08	23.18			
	T.S.	1345	1075	977	590			
3. Medium sandy	M.C.	24.80						
	T.S.	35,880						
4. Clyde F. S. loam	M.C.	*40.56	*43.82	41.91				
	T.S.	1320	1120	650				
5. Peat	M.C.	132.90	251.95					
	T.S.	1320	840					
6. Muck	M.C.	155.93	164.00					
	T.S.	1080	980					
7. Miami clay	M.C.	24.52						
	T.S.	260						

* Same soil. The moisture content of one was raised and allowed to stand for one week to come to an equilibrium. In No. 1 the last four are similar soils, while the other two are dissimilar but belong to the same type. Their total solids should not be compared with that of the others.

T. S., Total solids, parts per million of soil solution. M. C., Moisture content.

From Table I, it will be noted that the amount of moisture extracted depends upon the kind of soil and also the amount of organic matter present in the soil. The fine sandy soils with very little organic matter gave the best results, as seen in No. 4, where on the average over 60 per cent of the moisture present was extracted. In two or three instances over 70 per cent was extracted. The results obtained with No. 2 are excellent, considering the amount of moisture in the soil. The others varied considerably. In some cases this was due to improper packing, permitting the oil to come through before the extraction was completed. The data for peat and muck demonstrate how tenaciously the organic matter retains the water.

In the same types of soil, the increase in moisture content causes a decrease in total solids, measured in parts per million. In one or two cases it was noted that the total solids were inversely proportional to their moisture content. In most cases illustrating the above, the ratios

were not as would be desired, possibly because of the fact that the soils were not portions of the same sample and were taken at different times. The majority of soils extracted had from 700 to 1300 parts per million of solids. Several had nearly 2,000 and one as low as 260. One was exceptionally high, but it was a poor uuproductive soil. It had 35,880 parts per million and the freezing point was depressed 1.69°. Its other properties varied accordingly. Some of the reactions were neutral and others alkaline, as tested by means of the electric conductivity method.

The mineral constituents studied were those that were of some use to the plant, potassium, phosphoric acid, calcium (magnesium in later determinations), and nitrogen in the forms of ammonia, nitrite and nitrates. The nitrogen varied, but this is to be expected since it is a more changeable factor. The solutions from the same soil extracted at the same time, one from a small cylinder and the other from the larger one, in most cases, agreed fairly well in total nitrogen (sum of the different kinds), whereas they varied in the ammoniacal, nitrate, or nitrate nitrogen.

The phosphorus, especially in the various sandy soils, remained practically constant. The other types varied more or less. Table III gives an average of phosphorus, in parts per million, of the soil solutions, with the lowest and highest included in the average.

TABLE III

PHOSPHORUS IN SOLUTIONS FROM VARIOUS SOILS

Type of Soil	No. of Soils in Average	Phosphorus Average p.p.m.	Lowest p.p.m.	Highest p.p.m.
Sandy soils (including loams)..	21	4.3	3.0	5.20
Clay	1	9.2	...	....
Clay loam	2	15.7	6.8	25.00
Peat	4	8.8	1.6	15.40
Muck	2	2.6	2.5	2.70

The potash varied considerably in all the soils with the exception of peat and muck. It had a total range of 18 to 71 parts per million. In peat it varied from 71 to 125 parts per million, and in muck 20 to 38. The calcium and magnesium variation was quite marked. All the determinations were made by the colorimetric method, except for calcium and magnesium in the later work, which were made gravimetrically. A difference noted in the amount of nitrite present at the beginning and at the end of an extraction led to the examination physically and chemically of two similar soils, one a subsoil and the other a surface soil. Sandy soil was used because it gave a larger amount of solution for studying the various portions.

In the above two soils, successive portions of the solutions were examined as noted in Table IV. It will be noted that the successive por-

tions do not vary much in specific gravity or specific conductivity, but there is a slight decrease in total solids in Soil 34, while those in Soil 35 remain about the same. The difference here is more in the organic matter (loss on ignition) than in the inorganic matter. This would indicate that part of the total solids was adsorbed. The first portions came from the lower part of the cylinder and the last portion from the upper layers.

TABLE IV

PHYSICAL ANALYSES OF DIFFERENT PORTIONS OF SOLUTIONS FROM THE SAME SOIL

FINE SANDY SOIL (SUBSOIL)

Lab. No.	Total Moisture in Soil c.c.	Amount Extracted c.c.	Sp. Gravity	Sp. Conductivity	Parts per Million of Solution		
					Total Solids	Inorganic Matter	Organic Matter
34		538	1.000607	0.000863	1260	440	820
		450	1.000528	0.000889	1090	450	640
I	2710	445	1.000509	0.000901	790	340	450
		167	1.000528	0.000928	960	600	360
Total and average..		1600	1.000560	0.000887	933	432	501
		545	1.000607	0.000916	1100	660	440
		545	1.000490	0.000916	1280	420	860
		530	1.000528	0.000920	900	390	510
II	3828	540	1.000560	0.000904	880	540	340
		250	1.000512	0.000901	780	490	290
		225	1.000450	0.000904	790	550	240
		150	1.000560	0.000924	740	450	290
Total and average..		2785	1.000540	0.000913	981	507	474

FINE SANDY SOIL (SURFACE)

35	903	212	1.0010	0.000238	380	280	100
I		103	1.0022	0.000255	440	300	140
Total and average..		315	1.0017	0.000254	399	286	113
		200	1.00018	0.000257	440	300	140
II	1452	220	1.00010	0.000246	350	240	110
		95	1.00020	0.000274	430	330	100
Total and average..		515	1.00015	0.000257	400	280	120

I Small cylinder. II Large cylinder.

NOTE.—The averages are calculated according to the amount of moisture in the portions rather than by the number of determinations.

In Table V it will be noted in Soil 34 that the nitrogen as ammonia remains the same with one or two exceptions, but in Soil 35 there is a decrease. In all cases the nitrogen as nitrite increased while the nitrate decreased, with the exception of No. 35 II where it increased. The change in this case was between ammoniacal nitrogen and nitrate nitro-

gen. In nearly all cases there was a very slight decrease in the total nitrogen (sum of the different forms of nitrogen) in the successive portions of soil solution.

Only a very small percentage of the bacteria are removed from the soil since the soil in the cylinder acts as a filter in holding back most of the suspended substances. The soil acts on the same principle as a filter bed in a water-purification plant. If the cylinders are allowed to stand too long a very marked anaerobic decomposition takes place which is quite marked in clay soils or in those with considerable organic matter, as is evidenced by the disagreeable odor given off at the times of cleaning out the cylinders. In Table IV there will be noted a slight denitrification —less nitrates and more nitrites. This is especially true in Nos. 34 I and II, and 35 I.

TABLE V

NITROGEN DETERMINATION OF DIFFERENT PORTIONS OF THE SAME SOILS
(Amount of N in Various Portions of Extraction of a Sandy Soil)

Lab. No.	Portion	Parts per Million of Soil Solution Nitrogen as			
		NH_3	NO_2	NO_3	Total N
34	1	1.5529	Trace	1.6875	3.2403
	2	1.5528	0.0108	1.5000	3.0636
I	3	1.2930	0.1000	1.2300	2.8828
II	1	1.5528	Mere trace	1.7120	3.2640
	2	1.5528	Slight trace	1.5140	3.0660
	3	1.5528	Slight trace	1.4060	2.9580
	4	1.5528	0.0150	1.3340	2.9010
	5	1.5528	0.0550	1.1250	2.7320
	6	1.0352	0.1000	1.0440	2.1790
	7	1.3860	0.2390	0.8680	2.4930
35	1	0.5127	0.0111	0.3438	0.8676
I	2	0.2717	0.0291	0.3375	0.6383
II	1	0.5435	0.0062	0.3438	0.8935
	2	0.2939	0.0107	0.3938	0.6984
	3	0.2717	0.0112	0.4183	0.7012

I Small cylinder. II Large cylinder.

This soil solution is now being used in connection with the study of the microbial decomposition of nitrogenous compounds in the soil. Favorable progress has been made. Mr. O. M. Gruzit, in this laboratory, has used the soil solution to study the effect of some acids, alkalis and inorganic salts upon soil bacteria and has found some interesting results. A report on the resuts of these investigations is now being prepared for publication.

Summary

The paraffin oil pressure method furnishes in most cases plenty of solution for the necessary analytical work. In sandy soils as high as 74 per cent of the moisture present in the soils was obtained.

A large amount of solution may be obtained without its coming in contact with the oil. If it does it can be easily separated by cooling and by the separatory funnel.

The concentration of the soil solution from different samples of the same type of soil varies according to the moisture content of the samples from which it is derived.

Successive portions of the same extraction vary only slightly in their physical properties, but to a considerable extent in the various forms of nitrogen.

The forms of nitrogen vary in the different solutions, since they are changeable factors. Calcium and magnesium also vary according to the treatment and reaction of the soil. The phosphoric acid (PO_4) is fairly constant. Potash (K) varies somewhat.

A small percentage of the bacteria are removed from the soil, since the soil acts as a filter.

Anærobic changes take place in the cylinder if it is allowed to stand for a long time.

The paraffin oil displacement-pressure method furnishes, as far as we are able to judge with our present facilities, a fair representative of the solution as it exists in the soil. The method permits the use of a large amount of soil, thus a better representative sample. Work now in progress indicates that it furnishes a valuable index of the microbial changes in the soil.

Possibilities and Limitations

The soil solution is important from the plant physiologist's point of view for the studying of plant nutrients and balanced solutions. It opens another field for the soil chemist and physicist for the study of some of the soil conditions, *e. g.* composition and concentration of the liquid phases of the soil. To the soil bacteriologist it is important for the study of the microbial changes that takes place in the soil, of filterable micro-organisms in the soil and of the pathogenic microorganisms that will live in the soil.

The method has its limitations in that the oil does not entirely penetrate all soils and therefore, does not give all the soil solutions that might be otherwise obtained.

The obtaining of any solution is limited to the moisture content of the soil and depends upon the type of soil. The greater the moisture

content, the greater is the amount of solution that can be obtained from the various samples of similar soils. From samples of dissimilar soils having equal moisture contents, the proportion of the total moisture varies with the soil types, the largest percentage being obtainable from sandy soils and the smallest amount from clay soils.

The method is still open for improvement. Certain phases of it need further study, as the type of cylinder, displacing liquid, and the method of packing the soil in the cylinder.

Literature Cited

(1) Briggs, L. J.
1901. Filtration of suspended clay from soil solutions. U. S. Dept. Agr. Bur. Soils Bul. 19, p. 31.

(2) Briggs, L. J., and McCall, J. R.
1904. An artificial root for inducing capillary movement of soil moisture. *In* Science, n. s., no. 20, p. 566-568.

(3) Briggs, L. J., and McLane, J. W.
1907. The moisture equivalents of soils. U. S. Dept. Agr. Bur. Soils Bul. 45, p. 14, 23.

(4) Cameron, F. K.
1911. The Soil Solution. p. 32. Chemical Pub. Co., Easton, Pa.

(5) Cameron, F. K., and Bell, J. M.
1905. The mineral constituents of the soil solution. U. S. Dept. Agr. Bur. Soils Bul. 30.

(6) Cavers, F.
1914. Review of Gola's osmotic theory of edaphism. *In* Jour. Ecol., v. 2, no. 4, p. 204-231.

(7) Gedroitz, K. K.
1906. Solubility of soil compounds. *In* Zhur. Opuitn. Agron. (Russ. Jour. Expt. Landw.), v. 7, p. 529.

(8) Gola.
1911. Oswèrverzioni soprai liquidi circolanti nel terreno ogrario. *In* Ann. R. Accad. Agr. Torieno, v. 54. *Abs. in* Stiles and Jorgensen (16).

(9) Hilgard, E. W.
1912. Soils, their Formation Properties, Composition and Relations to Climate and Plant Growth in the Humid and Arid Regions. MacMillan Co., New York.

(10) Itscherekov, W.
1907. Obtaining soil solution in an unaltered condition. *In* Zuhr. Opuitn. Agron. (Russ. Jour. Expt. Landw.), v. 8, p. 147-165.

(11) Miller, N. H. J.
1906. The amount and composition of the drainage through unmanured and uncropped land, Barnfield, Rothamsted. *In* Jour. Agr. Sci., v. 1, no. 4, p. 377-399.

(12) Rayleigh, Lord.
1890. On the theory of surface forces. *In* Phil. Mag. and Jour. Sci., v. 5, p. 30, 285-298, 456-475.

(13) SCHLOESING, TH.
1866. Sur l'analyse des principes soluble de la terre vegetable. *In* Compt. Rend. Acad. Sci. (Paris), t. 63, p. 1007.

(14) SCHLOESING, TH.
1870. Analyse des eaux contenues dans les terres arable. *In* Compt. Rend. Acad. Sci. (Paris), t. 70, p. 98, 99.

(15) SCHREINER, O., and FAILYER, G. H.
1905. Colorimetric, turbidity and titration methods used in soil investigation methods. U. S. Dept. Agr. Bur. Soils Bul. 31, p. 16-17.

(16) STILES, W., and JÖRGENSEN, I.
1914. Nature and method of extraction of the soil solution. *In* Jour. Ecol., v. 2, no. 4, p. 249.

(17) VAN SUCHTELEN, F. H. H.
1912. Methodi zur Gewinning der naturlichen Boden Lösung. *In* Jour. Landw., Bd. 60, p. 369-370.

(18) ZACHAROV, S. A.
1906. Soil solutions: Their rôle in the soil formation, methods of their examination and their significance for the characterization of soil types. *In* Zhur. Opuitn. Agron. (Russ Jour. Expt. Landw.), v. 7, p. 388-477.

PLATE I

Fig. 1.—Battery of cylinders showing how connected and means of collecting soil solutions.

Fig. 2.—Cylinder and its parts.

A, cylinder; B, wire gauze; C, cylinder; D, perforated iron plate; E, pedestal with spout; F, copper and asbestos gasket.

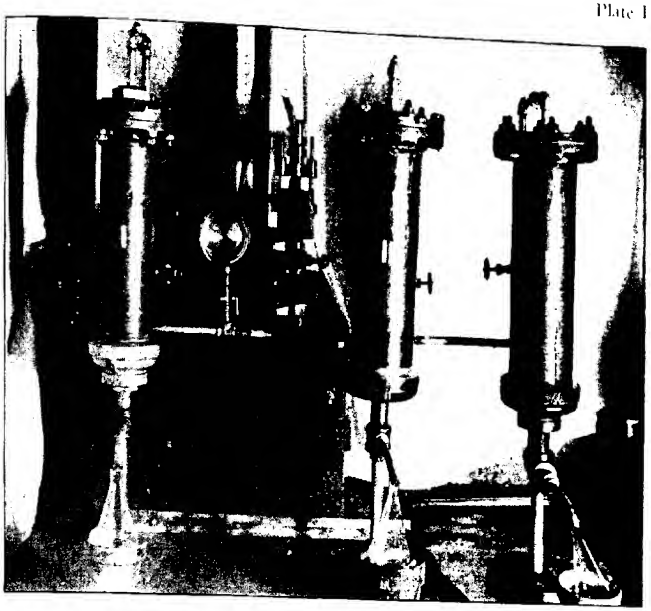

Fig. 1.

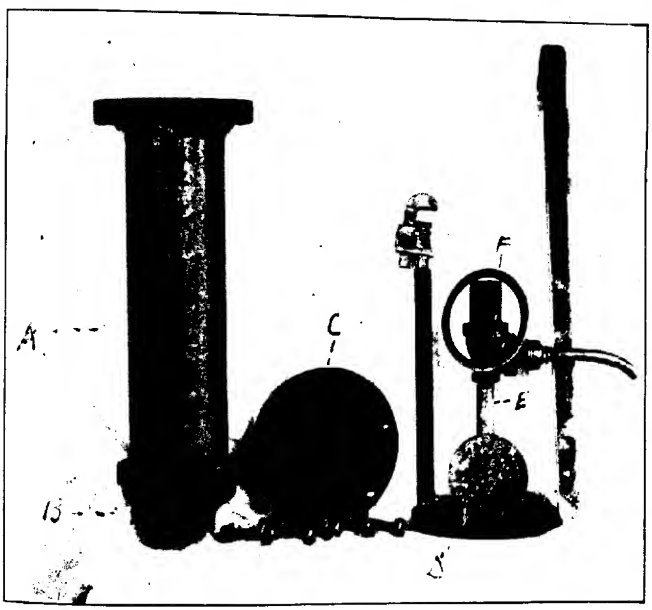

Fig. 2.

THE EFFECT OF HYDROGEN AND HYDROXYL ION CONCENTRATION ON THE GROWTH OF BARLEY SEEDLINGS[1]

By

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Introduction

There now exists an extensive literature (8) concerning the relation of hydrogen ion concentration to various biological processes. The importance of accurately measuring the concentration of the hydrogen ion in studies on bacterial metabolism, enzyme hydrolyses, changes in tissue fluids, etc., is well recognized. But comparatively few experiments, however, have been recorded which bear directly on the growth of plants of agricultural importance as affected by the acidity or alkalinity of the media. In most of the experiments, moreover, there has been no measurement or control of the hydrogen ion concentration. The reaction has been regulated by titration methods, or by the use of dilute solutions of highly dissociated acids or bases, often implying an excessive concentration of the hydrogen or hydroxyl ion. From a biological and agricultural standpoint it is of much greater value to investigate the behavior of the plant when it is subjected to definite variations of hydrogen ion concentration within not too wide limits on each side of the neutral point. The concentration of the hydrogen ion in even very dilute HCl solution, for example, may be far greater than can possibly occur in acid soils or nutrient solutions, and it is just these latter conditions which need more extensive study.

Recently Gillespie (5), and Sharp and the author (15) have measured the H-ion concentrations of various soil suspensions, and have thus determined approximately the magnitudes of the hydrogen ion concentrations in various soils of acid, alkali, and slightly alkaline types. The purpose of the present investigation has been to study the effect of similar hydrogen and hydroxyl ion concentrations in nutrient media, where the many other variables of the soil can be eliminated. Only in this way is it possible to understand the specific influence of the hydrogen and hydroxyl ion on the growth of the plant. It seems more convenient first to describe the results obtained in this laboratory, and later to discuss the previous literature with its bearing on the specific experiments and its relation to the general subject.

[1] Received for publication April 3, 1917.

Disscussion of Experiments

In order to maintain definite concentrations of the hydrogen or hydroxyl ion, solutions must be used which have a reserve of acidity or alkalinity, the so-called "buffer" solutions. In the present instance it was necessary to use solutions which should be nutrient for plants and non-toxic. The various potassium phosphates seemed precisely adapted to this purpose. Any desired concentration of hydrogen or hydroxyl ion could be obtained by the use of suitable mixtures of the mono, di, and tri-basic phosphates. A complete nutrient solution would contain in addition to potassium, phosphorus, and nitrate, also iron, calcium and magnesium, but such solutions are difficult or impossible to prepare when the OH-ion concentration exceeds a certain minimum, because of the precipitation of insoluble phosphates. It was therefore decided to adopt partial nutrient solutions, omitting calcium, magnesium and iron. This is not a serious disadvantage during the first stages of growth, when the plant may still obtain the missing elements from the store in the seed. The desirability of such a procedure lies in the possibility of making comparisons throughout the whole range of H and OH-ion concentrations, in solutions otherwise similar in composition, and having like osmotic pressures.

The usual methods of water culture experiments were followed. Barley seeds were sprouted between layers of ordinary wet paper toweling, a method suggested by Dr. Waynick of this college. This technique was very successful. Uniform seedlings were obtained entirely free from all fungous growths. In all tests selected barley seeds of the Beldi variety were used. Care was taken to select seeds of uniform size. When the seedlings were about ¾ inch long, they were transferred to bottles of 150-c.c. capacity, containing the experimental solutions. The bottles were painted on the outside with black asphalt paint to prevent injury to the roots by light. Flat corks, in which small holes were bored, served to support the seedlings and small wads of cotton were used to fix them in place.

The experiments were conducted in successive series. Each series consisted of three sets of solutions, which always included, for comparison, one solution with a neutral or slightly alkaline reaction. For each solution 8 bottles were used, with 3 plants in each bottle. The cultures were usually placed outside in the sunshine during the day, and at night were kept in a fume-free room. Every effort was made to have the results strictly comparable for each series. Vigorous, uniform growth was secured in all the favorable solutions. The seedlings were grown for 14 days.

Experiments with Alkaline Solutions

The first experiments were planned to furnish data which might indicate the effect of various concentrations of OH-ion in alkaline solutions. The OH-ion concentration was adjusted by using different proportions

of K_3PO_4 and K_2HPO_4. The nutrient solutions were made by dilution of approximately M/4 or M/2 solutions. The latter were prepared by weighing out the appropriate quantities of Baker's analyzed salts. These solutions gradually attack glass, and new solutions were made up at frequent intervals. In the later series, in addition to the phosphates, small proportions of $NaNO_3$, Na_2SO_4, and NaCl were used. It was thought that in this way a better balance might possibly be obtained.

The hydrogen ion concentration of all solutions was ascertained by electrometric measurement with a Hildebrand hydrogen electrode apparatus (15). It has been shown that NO_3 is reduced by H gas in the presence of platinum black. Upon investigation, however, it was found that in the "buffer" phosphate mixtures, and employing the shaking method described by Clark and Lubs (3), no appreciable error was introduced through NO_3 reduction. As evidence thereof, similar phosphate mixtures with and without $NaNO_3$ present, gave identical readings under the experimental conditions. The accuracy of the electrometric readings was approximately .005 volt. Greater precision would be useless in investigations of this character. Voltmeter readings were converted into H-ion concentrations with the aid of tables calculated by Schmidt (14).

In the first series total concentrations of approximately 3500 parts per million of total solids were used, but in the later series a concentration of 2200 parts per million was adopted, as being more favorable to growth. In each series the total concentrations of the different solutions were made very similar in order to eliminate effects of osmotic pressure from the comparisons. Freezing-point depressions were determined on a number of the solutions of the later series, and the results were found to be very uniform, approximately 0.06° C. lowering, equivalent to 0.7 atmosphere.

In the first series the distilled water used in preparing the nutrient solutions was treated with carbon black. This treatment was later discarded, since no evidence of toxicity was noted. The distilled water was obtained from a supply stored in a heavily tinned reservoir.

Table I gives the essential data concerning the composition of the alkaline solutions of the various series.

After the seedling had grown in such solutions for several days, the OH-ion concentration diminished very appreciably, for reasons to be discussed later. In order to maintain the reaction at an approximately constant value, the solutions were changed frequently, in most cases every day. The table shows, then, the range of OH-ion concentrations to which the plants were subjected during periods of 24 to 48 hours.

The effect of the various solutions on the seedlings was determined from the following criteria; general appearance of roots and tops, development of lateral roots and root hairs, dry weights (at 100° C.) of tops, roots and residual seeds. In most of the experiments the fresh weight and the average length of the tops also were noted. In a num-

TABLE I

COMPOSITION OF ALKALINE SOLUTIONS

(c.c. of Strong Solutions added to 1 liter of Nutrient Solution)

No. of Nutrient Solution	K_2PO_4 M/4 c.c.	K_2HPO_4 M/2 c.c.	$NaNO_3$ M/2 c.c.	NaCl M/1 c.c.	Na_2SO_4 M/2 c.c.	Approximate Total Concentration p.p.m.	Concentration H-ion
			SERIES I				
1	17.0	30.0	2.5	...	...	3600	0.45×10^{-7}
2	40.0	17.3	2.5	...	...	3700	0.65×10^{-9}
3	46.0	10.0	2.5	...	...	3400	0.34×10^{-10}
			SERIES II				
4	8.5	15.0	2.5	...	...	1800	0.45×10^{-7}
5	20.0	8.6	2.5	...	...	1900	0.65×10^{-9}
6	23.0	5.0	2.5	...	...	1700	0.40×10^{-10}
			SERIES III				
7	8.5	15.0	2.5	0.8	0.8	2200	0.35×10^{-7}
8	20.0	8.6	2.5	0.8	0.8	2200	0.16×10^{-9}
9	23.0	5.0	2.5	0.8	0.8	2100	0.14×10^{-10}
			SERIES IV				
10	8.5	15.0	2.5	0.8	0.8	2200	0.42×10^{-7}
11	20.0	8.6	2.5	0.8	0.8	2200	0.16×10^{-9}
12	23.0	5.0	2.5	0.8	0.8	2100	0.14×10^{-10}
			SERIES V				
13	7.8	15.0	1.7	0.8	0.8	2100	0.30×10^{-7}
14	16.6	9.1	1.7	0.8	0.8	2100	0.41×10^{-8}
15	18.3	6.8	1.7	0.8	0.8	1900	0.10×10^{-8}
			SERIES VI				
16	....	19.0	1.7	0.8	0.8	2000	0.78×10^{-7}
17	16.6	9.1	1.7	0.8	0.8	2100	0.41×10^{-8}
18	18.3	6.8	1.7	0.8	0.8	1900	0.16×10^{-8}
			SERIES VII				
19	....	19.0	1.7	0.8	0.8	2000	0.78×10^{-7}
20	16.6	9.1	1.7	0.8	0.8	2100	0.30×10^{-8}
21	18.3	8.3	1.7	0.8	0.8	1900	0.17×10^{-9}
			CONTROL SERIES				
22	....	19.0	1.7	0.8	0.8	2000	0.80×10^{-7}
22	....	19.0	1.7	0.8	0.8	2000	0.80×10^{-7}

In each series the first solution is considered as a control and is neutral or very slightly alkaline in reaction.

ber of cases microscopic examinations of the roots were made. The data given in Table II will aid in comparing the effects of the different solutions.

The results in Table II are entirely consistent in demonstrating that increased OH-ion concentration is accompanied by appreciable changes in most of the observed factors. That the variations are not accidental is made quite clear by the control experiment. In this case two sets of plants were grown in neutral solutions under the experimental conditions employed in all series. The duplicate results are in excellent agreement. The probable error due to individuality of plants in these sets is apparently negligible.

The general effect of the higher concentrations of OH-ion has been markedly to decrease the fresh and dry weights of the tops, and the average length. The development of lateral roots has been almost entirely repressed. Microscopic observations on the root tips of seedlings grown in the higher concentrations of OH-ion indicated unquestionable injury to the root tips. In many cases the leaves also gave evidence of toxicity by a wilted appearance and yellowing of the tips.

The inference seems to be justified that OH-concentrations greater than approximately 1.8×10^{-6} cause injury to the seedling, and that concentrations greater than 2.5×10^{-5} are extremely toxic, either through direct action on the cells or indirectly by some disarrangement of the metabolic processes. That sodium carbonate in concentrations of 1000 parts per million very greatly reduces the absorption of potassium and phosphoric acid by wheat seedlings has recently been shown by Breazeale (1).

Effect of Hydrogen Ion in Acid Solutions

So far only neutral or alkaline solutions have been considered. What would be the effect of similar solutions having a concentration of H-ion greater than that of a neutral solution? The experiments carried out to answer this question were in all respects similar to those already described. The increased H-ion concentration was obtained by the use of KH_2PO_4, supplemented in one set by a suitable amount of 1 per cent H_3PO_4. In this way "buffer" mixtures were obtained with the desired intensities of acidity. The solutions used are given in Table III.

The effects of these solutions on the growth of the seedlings are shown in Table IV.

It is evident from these data that an acid condition is favorable to growth of the seedlings, in concentrations as high as 0.7×10^{-5} H-ion. There was an increase in the fresh and dry weight of the tops and the average length, as compared with the neutral solutions. The development of lateral roots was especially good, and microscopic examination gave no evidence of injury to the root tips. Tottingham's (7) work substantiates these conclusions. He found that wheat seedlings grown for 42 days in complete nutrient solutions gave a considerable increase in dry weight when KH_2PO_4 was used instead of K_2HPO_4. While he did not

TABLE II

DATA ON PLANTS GROWN IN ALKALINE SOLUTIONS

No. of Nutrient Solution	H-ion Concentration		Fresh Weight of Tops gm.	Dry Weights			Aver. Length of Tops cm.	Relative Values of Dry Weights of Tops	Root Development	Remarks
	Original Solution	Average at Time of Changing Solution		Tops gm.	Roots gm.	Residual Seeds gm.				
SERIES I										
1	0.45×10^{-7}	0.45×10^{-7}	....	0.330	0.110	0.310	....	100	Some lateral roots	
2	0.65×10^{-9}	0.30×10^{-8}	....	0.270	0.110	0.330	....	82	Very few lateral roots	Injury to tops
3	0.34×10^{-10}	0.14×10^{-9}	....	0.260	0.090	0.330	....	79	Very few lateral roots	Injury to tops
SERIES II										
4	0.45×10^{-7}	0.45×10^{-7}	....	0.410	0.180	0.330	....	100	Many laterals	
5	0.65×10^{-9}	0.91×10^{-8}	....	0.300	0.140	0.330	....	73	Smaller number of laterals	Some injury to tops
6	0.40×10^{-10}	0.98×10^{-9}	....	0.280	0.120	0.330	....	68	Very few laterals	Some injury to tops
SERIES III										
7	0.35×10^{-7}	0.35×10^{-7}	....	0.330	0.130	0.310	11.6	100	Many laterals	
8	0.16×10^{-9}	0.28×10^{-8}	....	0.330	0.130	0.340	11.5	100	Very few laterals	
9	0.14×10^{-10}	0.90×10^{-10}	....	0.270	0.120	0.330	10.0	82	Very few laterals	Roots discolored, tops injured
SERIES IV										
10	0.42×10^{-7}	0.42×10^{-7}	3.72	0.480	0.170	0.330	12.0	100	Many laterals	
11	0.16×10^{-9}	0.28×10^{-8}	3.16	0.420	0.170	0.330	11.5	88	Very few laterals	
12	0.14×10^{-10}	0.90×10^{-10}	2.82	0.360	0.140	0.310	10.6	75	Very few laterals	

					SERIES V					
13	0.30×10^{-7}	0.30×10^{-7}	3.62	0.360	0.140	0.290	9.9	100	Many laterals	Unfavorable weather during this series
14	0.41×10^{-8}	0.90×10^{-8}	3.07	0.310	0.140	0.340	9.5	85	Very few laterals	
15	0.10×10^{-8}	0.50×10^{-6}	3.42	0.320	0.160	0.310	9.7	89	Very few laterals	
					SERIES VI					
16	0.78×10^{-7}	0.78×10^{-7}	3.72	0.410	0.140	0.280	10.7	100	Many laterals	
17	0.41×10^{-8}	0.10×10^{-7}	2.98	0.330	0.140	0.290	9.6	80	Very few laterals	
18	0.16×10^{-8}	0.80×10^{-8}	3.26	0.340	0.150	0.290	10.0	83	Very few laterals	
					SERIES VII					
19	0.78×10^{-7}	0.78×10^{-7}	3.82	0.440	0.140	0.300	10.5	100	Many laterals	
20	0.30×10^{-8}	0.10×10^{-7}	3.30	0.380	0.135	0.290	10.0	86	Few laterals	
21	0.17×10^{-9}	0.28×10^{-8}	3.07	0.350	0.130	0.290	9.7	80	Almost no laterals	Leaf injury marked
					CONTROL SERIES					
22	0.80×10^{-7}	0.80×10^{-7}	4.31	0.495	0.160	0.265	10.8	100	Root development same in both	
22	0.80×10^{-7}	0.80×10^{-7}	4.27	0.495	0.165	0.275	10.8	100		

EXPLANATION:—

All calculations are on a basis of 24 plants. In a very few cases it was necessary to discard individual plants, due to accidental injury, not connected with the properties of the solution.

In calculating the relative weights of tops, the neutral or slightly alkaline solution in each series is taken as 100.

The average changes in H-ion concentration of the neutral solutions was within the limit of error and they are recorded as showing no change.

correlate this specifically with H-ion concentration, solutions similar to his have been measured electrometrically in this laboratory, and found to give an H-ion concentration of about 1.0×10^{-5} when KH_2PO_4 was used.

When the H-ion concentration was increased to 0.3×10^{-3} as in solution No. 31, then very decided injury was caused. There was a large decrease in dry weight, and the roots were unhealthy in appearance and produced no lateral roots. Microscopic examination at the end of one week's growth showed the root tips to be dead. The root hairs were abundant, however. There was even evidence of stimulation in this direction. The leaves were distinctly injured, having a scorched appearance at the tips.

TABLE III
COMPOSITION OF ACID SOLUTIONS
(c.c. of Strong Solutions added to 1 liter of Nutrient Solution)

No. of Nutrient Solution	K_2HPO_4 M / 2 c.c.	KH_2PO_4 M / 2 c.c.	$NaNO_3$ M / 2 c.c.	NaCl M / 1 c.c.	Na_2SO_4 M / 4 c.c.	Approximate Total Concentration p.p.m.	H-ion Concentration
SERIES VIII							
23	19.0	....	1.7	0.8	0.8	2000	0.85×10^{-7}
24	11.0	11.0	1.7	0.8	0.8	2100	0.20×10^{-6}
25	....	25.0	1.7	0.8	0.8	2100	0.75×10^{-6}
SERIES IX							
26	19.0	....	1.7	0.8	0.8	2000	0.85×10^{-7}
27	11.0	11.0	1.7	0.8	0.8	2100	0.26×10^{-6}
28	....	25.0	1.7	0.8	0.8	2100	0.69×10^{-5}
SERIES X							
29	19.0	....	1.7	0.8	0.8	2000	0.85×10^{-7}
30	....	25.0	1.7	0.8	0.8	2100	0.65×10^{-5}
*31	....	10.0	1.7	0.8	0.8	1200	0.29×10^{-3}

* + 10 c.c. 1% H_3PO_4.

All the foregoing results lead to the conclusion that any very appreciable increase in OH-ion concentration produces injury, while an equal degree of increase in H-ion concentration may be favorable, although, as just shown, a very great increase in H-ion concentration is also extremely detrimental to the plant. Some previous investigators have reached conclusions opposite to these, and it will now be desirable briefly to discuss these differences.

Breazeale and Le Clerc (2) investigated the effect of acid and alkaline media upon the growth of wheat seedlings. Solutions of dilute H_2SO_4 and HCl were used, also solutions of K_2SO_4, KCl and $NaNO_3$. It was assumed that in the case of the K_2SO_4 and KCl the acid condition found was due to selective absorption of the K-ion and that $NaNO_3$ gave an alkaline solution because the NO_3-ion was absorbed more rapidly than the Na-ion. The acid solutions were found to be extremely toxic, the alkaline solutions much less so. In interpreting these experiments it should be recalled that even in the small quantities used HCl or H_2SO_4 would

TABLE IV

DATA ON PLANTS GROWN IN ACID SOLUTIONS

No. of Nutrient Solution	H-ion Concentration		Fresh Weight of Tops gm.	Dry Weights			Aver. Length of Tops cm.	Relative Values of Dry Weights of Tops	Root Development
	Original solution	Average at Time of Changing Solution		Tops gm.	Roots gm.	Residual Seeds gm.			
SERIES VIII									
23	0.85×10^{-7}	0.85×10^{-7}	4.10	0.430	0.150	0.280	11.4	100	Many laterals
24	0.20×10^{-6}	0.20×10^{-6}	4.25	0.440	0.150	0.280	12.0	103	Increased number of laterals
25	0.75×10^{-6}	0.30×10^{-6}	4.63	0.470	0.160	0.300	12.6	109	Increased number of laterals
SERIES IX									
26	0.85×10^{-7}	0.85×10^{-7}	3.92	0.460	0.160	0.300	10.4	100	Many laterals
27	0.26×10^{-6}	0.26×10^{-6}	4.32	0.490	0.160	0.290	10.9	107	Increased number of laterals
28	0.69×10^{-6}	0.30×10^{-6}	4.38	0.490	0.160	0.280	11.0	107	More laterals than in solutions 26 or 27
SERIES X									
29	0.85×10^{-7}	0.85×10^{-7}	3.75	0.440	0.150	0.300	10.6	100	Excellent root development
30	0.65×10^{-5}	0.26×10^{-5}	4.40	0.490	0.170	0.310	11.4	111	Excellent root development
31	0.29×10^{-3}	0.16×10^{-3}	3.34	0.340	0.110	0.280	10.3	77	Roots unhealthy, no laterals—root tips dead

give excessive concentrations of H-ion. For example even a 1/2000 normal HCl solution yields a higher H-ion concentration than was found to be toxic in the experiments described in this paper. On the other hand, a very dilute NaOH solution, while having temporarily a high concentration of OH-ions, would rapidly decrease in alkalinity because of the action of CO_2, given off by the plant roots or present in the solution. Thus, with the formation of HCO_3-ions and a slight excess of free CO_2 the reaction would be not far from the neutral point, and so deductions with regard to the effect of the OH-ion might be entirely invalidated. It would not be necessary to conclude from such experiments that the H-ion is more toxic than the OH-ion.

In order to confirm these hypotheses, several culture experiments were carried out in this laboratory. Barley seedlings were grown in the usual manner in solutions of KOH (1/2000 normal), HCl (1/2000 normal), and $KHCO_3$ (500 parts per million). The HCl solutions were fatal to the plants, while neither the KOH nor $KHCO_3$ solutions gave any indications of injury. The root development in both cases was in fact better than in the most favorable solutions previously examined. Microscopic examination showed no injury to the root tips. The results are entirely dissimilar to those obtained in solutions having a high OH-ion concentration. Actual electrometric measurements of $KHCO_3$ solutions, in equilibrium with a small quantity of CO_2 gas, showed a neutral or slightly acid condition. All these considerations are confirmatory of the conclusion that such dilute solutions of KOH or NaOH are not capable of giving information concerning the effect of the OH-ion on plant growth. Where high concentrations of Na_2CO_3 are present, as in the case of some soils, then it is probable that there exists constantly an injurious concentration of OH-ions. Practical field observations accord with this view.

Miyake (9), working on the rice plant, grew seedlings in H_2SO_4 and HCl solutions of 1/10 to 1/20,000 normal and in KOH and NaOH solutions from 1/10 to 1/10,000 normal. He reaches the general conclusion that the H-ion is much more toxic than the OH-ion. Hartwell and Pember (7) experimented on various cereals, growing in dilute KOH, HCl and H_2SO_4 solutions, and drew similar deductions. Dachnowski (4) conducted experiments on tomato cuttings and found 1/800 normal KOH solutions to be beneficial, while acid solutions were very harmful. It is obvious that the difficulties of interpretation already mentioned would apply also in all these experiments.

That the H-ion may not necessarily be so toxic to plants as is sometimes assumed has been idicated by Haas (6) in a recent paper. This investigation gave evidence that the cell sap of certain plants may have a very considerable concentration of H-ion.

From a practical standpoint the question of the relation of H-ion concentration to plant growth is of great importance because of the prevalence of acid soils in certain localities, and of "alkali" soils in arid regions. It would appear from these experiments that acid soils with an H-ion concentration of 0.8×10^{-5} or less would not be injurious to plants of the cereal group because of excessive acidity. Final conclusions on this point must be reserved until observations are made of the entire period of growth of the plant. In any case, many toxic factors, other than the H-ion may be present in acid soils. The aluminum ion has been shown to be extremely toxic in moderate concentrations (10, 13). Under acid conditions, its solubility would presumably be greatly increased. There are also to be considered of course the possible presence of toxic organic compounds, lack of plant-foods, inhibition of bacterial activities, bad physical conditions, etc. All of these injurious influences might be alleviated by the use of lime, in addition to its action in neutralizing acidity. While it is extremely probable that some plants are far more susceptible to injury by the H-ion than others, yet no conclusions of this sort can safely be drawn until the effect of the H-ion is studied apart from other injurious factors, and it would seem difficult or impossible to obtain the requisite conditions in the acid soil itself. Similar considerations would apply to alkali soils. Here possibly antagonistic effects of other ions might serve to decrease injury, in the general manner described by Osterhout (11). It is very doubtful, however, whether any combination of ions could greatly lessen the injurious action of a high concentration of OH-ions, except by actually decreasing their concentration, while the injury might be accentuated by an excessive osmotic pressure.

The Change in Reaction in the Solutions by the Seedling

Early in this investigation it was observed that after one or more days, the culture solutions had in some cases very appreciably changed their H-ion concentration, as determined by actual measurement. It was thought that one explanation might be the reaction between the solutions and the glass of the bottles. Solutions were permitted to remain for several days in culture bottles, in the absence of seedlings. Slight changes in H-ion concentration were noted, but of a very much less magnitude than when the plants were growing in the solutions. In general it was found that the alkaline solutions decreased markedly in OH-ion concentration, acid solutions decreased slightly in H-ion concentration, while neutral solutions remained practically constant. This must be the result either of the secretion of neutralizing substances by the plant, of chemical reaction with the material of the roots or of the selective absorption of specific ions.

Some typical examples of the changes in reaction are given in Table V.

These data would seem to point to a general tendency by the plant so to regulate the reaction of the media that excessive concentrations of H or OH-ion can not occur. Breazeale and Le Clerc (2) in their experiments reached a somewhat contrary conclusion, since they found that KCl and K_2SO_4 solutions became sufficienty acid distinctly to injure wheat seedlings, especially the roots. A number of cultures of barley seedlings grown in this laboratory in KCl solutions of 500 parts per million total concentration gave no evidence of injury due to excessive H-ion concentration. Electrometric measurements on the solutions at several periods during the growth of the plants gave H-ion concentrations of only about 0.2×10^{-4}. If HCl had been formed as a result of selective absorption of the K-ion, it would require approximately only 0.7 part per million of HCl to produce this H-ion concentration. The actual total residual acid found by Breazeale and Le Clerc was in some cases 14 parts per million,

TABLE V

CHANGES IN H-ION CONCENTRATION OF SOLUTIONS DUE TO GROWTH OF PLANTS

No. of Nutrient Solution	H-ion Concentration at Start	H-ion Concentration at Time of Changing	Time Interval Days
2	0.65×10^{-9}	0.28×10^{-8}	2
3	0.34×10^{-10}	0.13×10^{-9}	2
5	0.65×10^{-9}	0.12×10^{-7}	3
6	0.40×10^{-10}	0.48×10^{-8}	3
8	0.16×10^{-9}	0.28×10^{-6}	2
9	0.14×10^{-10}	0.82×10^{-10}	2
25	0.75×10^{-6}	0.29×10^{-6}	3
30	0.65×10^{-6}	0.26×10^{-6}	4
31	0.29×10^{-3}	0.16×10^{-3}	4
10	0.42×10^{-7}	0.45×10^{-7}	3
13	0.30×10^{-7}	0.38×10^{-7}	1
16	0.78×10^{-7}	0.78×10^{-7}	1
16	0.78×10^{-7}	0.92×10^{-7}	3

and similar results were obtained in the present investigation. The data obtained with the hydrogen electrode would not point to the presence of a highly dissociated acid in any appreciable quantity. The acidity might be due to organic acids. Recently Pantanelli (12) has made very extensive studies of the selective absorption of ions by plants. He finds that in nearly all cases certain ions are absorbed more rapidly than others, when the plant is grown in solutions of single salts, although the diffusion of other ions from the plant into the solution is not excluded. Incidental to this work Pantanelli studied the changes of H-ion concentration of the culture solutions, and finds evidence that the plant tends to maintain a favorable H-ion concentration.

It seems possible in certain experiments with KCl and K_2SO_4 solutions, that other factors than excessive acidity may operate to produce toxic effects. Frequently aluminum discs are used to support the seedlings. The toxicity of the aluminum ion has already been referred to,

and it is suggested that sufficient aluminum might be dissolved in slightly acid solutions to injure the seedling. In order to test this hypothesis a number of barley seedlings were grown in KCl solutions and also in similar KCl solutions to which a small piece of aluminum foil had been added. No differences were noted in the appearance of the tops but the roots in the solutions containing the aluminum foil, when examined microscopically, showed injury to the root tips. No lateral roots were produced when aluminum was present, while in the control KCl solutions, numerous laterals in an incipient stage had appeared. The data given here with regard to the toxic action of aluminum are only preliminary in nature, but this incidental phase of the investigation is mentioned in order to suggest the possible interference of a complicating factor in similar studies of selective adsorption and of changes in the H-ion concentration of the solution caused by the plant.

Summary

1. Barley seedlings were grown in partial nutrient solutions of like osmotic pressure, but having a considerable range of H and OH-ion concentrations, produced by the use of the various potassium phosphates. The H-ion concentrations were controlled in all cases by hydrogen electrode measurements.

2. The OH-ion in such solutions was found to be more toxic than the H-ion for similar divergences from the neutral point.

3. For the solutions used a concentration of OH-ion greater than 1.8×10^{-6} was distinctly injurious, and when exceeding 2.5×10^{-5} extremely toxic.

4. A concentration of H-ion of approximately 0.7×10^{-5} was found to be favorable to growth and to produce no injury. A concentration of 0.3×10^{-3} was very toxic.

5. The relation of the data to acid and alkali soils and to the question of selective absorption is briefly discussed. Reference is made to the toxicity of aluminum.

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Literature Cited

(1) Breazeale, J. F.
1916. Effect of sodium salts in water cultures on the absorption of plant food by wheat seedlings. *In* Jour. Agr. Research, v. 7, no. 9, p. 407-416.

(2) Breazeale, J. F., and Le Clerc, J. A.
1912. The growth of wheat seedlings as affected by acid or alkaline conditions. U. S. Dept. Agr. Bur. Chem. Bul. 149.

(3) CLARK, W. M., and LUBS, H. A.
1916. Hydrogen electrode potentials of phthalate, phosphate, and borate buffer mixtures. *In* Jour. Biol. Chem., v. 25, no. 3, p. 479-510.

(4) DACHNOWSKI, A.
1914. The effects of acid and alkaline solutions upon the water relation and the metabolism of plants. *In* Amer. Jour. Bot., v. 1, p. 412-439.

(5) GILLESPIE, L. J.
1916. The reaction of soil and measurements of hydrogen-ion concentration. *In* Jour. Wash. Acad. Sci., v. 6, no. 1, 7-16.

(6) HAAS, A. R.
1916. The acidity of plant cells as shown in natural indicators. *In* Jour. Biol. Chem., v. 27, no. 1, p. 233-241.

(7) HARTWELL, B. L., and PEMBER, F. R.
1907. The relation between the effects of liming, and of nutrient solutions containing different amounts of acid, upon the growth of certain cereals. *In* R. I. Agr. Exp. Sta. Ann. Rpt., v. 20, p. 358-380.

(8) MICHAELIS, L.
1914. Die Wasserstoff ionenkoncentration. Berlin.

(9) MIYAKE, K.
1914. Ueber die Wirkung von Saüren, Alkalien und einiger Alkali auf dem Wachstum der Reispflanzen. *In* Trans. Sapporo Nat. Hist. Soc., v. 5, pt. 2, p. 91-95.

(10) MIYAKE, K.
1916. Toxic action of soluble aluminum salts upon the growth of the rice plant. *In* Jour. Biol. Chem., v. 25, no. 1, p. 23-28.

(11) OSTERHOUT, W. J. V.
1914. The effect of alkali on permeability. *In* Jour. Biol. Chem., v. 19, p. 335-343.

(12) PANTANELLI, E.
1915. Absorption of ions by plants. *In* Jahrb. Wiss. Bot. (Pringsheim), Bd. 56, p. 689-733.

(13) RUPRECHT, R. W.
1915. Toxic effects of iron and aluminum salts on clover seedlings. *In* Mass. Agr. Exp. Sta. Bul. 161, p. 125-129.

(14) SCHMIDT, C. L. A.
1909. Table of H+ and OH— concentrations corresponding to electrometric forces determined in gas chain measurements. *In* Univ. Cal. Pub. Physiol., v. 3, no. 15, p. 15, p. 101-113.

(15) SHARP, L. T., and HOAGLAND, D. R.
1916. Acidity and adsorption in soils as measured by the hydrogen electrode. *In* Jour. Agr. Research, v. 7, no. 3, p. 123-145.

(16) TOTTINGHAM, W. E.
1914. A quantitative chemical and physiological study of nutrient solutions for plant cultures. *In* Physiol. Researches, v. 1, no. 4, p. 133-245.

ADSORPTION OF AMMONIUM SULFATE BY SOILS AND QUARTZ SAND[1]

PRELIMINARY COMMUNICATION

BY

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One of the most important questions in soil fertility is that of the soil solution and the factors modifying it. On account of the nature of the soil constituents, origin of the soil, and its chemical composition, soil adsorption is one of the factors which cause the modification of the soil solution both qualitatively and quantitatively. Hence, the urgent need of studying the different phases of this important problem is evident to every careful student of soil fertility.

Without even briefly reviewing the results of previous investigations on this question, which will be published elsewhere, together with the complete data of the present work, the author wishes to present here a brief summary of the results obtained on the adsorption of ammonium sulfate by different soils and also by quartz sand of different degrees of fineness. The method employed in testing the concentration of the soil solution was the freezing-point depression method, as outlined by Bouyoucos and McCool (1). Six different soils were used: a medium sandy loam, a medium loam, a medium silt loam and a heavy silt loam of the Sassafrass series, Penn shaley loam and muck. Quartz sand used was of 24-mesh, 60-mesh, 124-mesh, and 5/0 and 7/0 bolting cloth. The ammonium sulfate solutions used were of the following concentrations: N/2, N/4, N/8, N/16, N/32, N/64, N/128 and N/256. The studies were made on the adsorption of salt by the soil as modified by (a) the concentration of the salt solution, (b) organic matter, (c) previous treatment with different fertilizers, (d) temperature, (e) time allowed for reaction, and (f) the water content in the soil.

The procedure of the experiments, in general, was as follows: A given amount of soil, usually 20 gm., was treated with a certain amount of distilled water, or the ammonium sulfate solution of a given strength, mixed thoroughly, placed into a test tube, stoppered and left over night in a chamber of practically constant temperature and of saturated atmosphere. On the following morning, without taking the contents out of the tube, the freezing point of the mixture was determined. The freezing-point depression of the soil due to the salt solution applied was always compared with that of the soil treated with distilled water, which was used as a check. Every fourth or fifth determination was of distilled water alone, this being the check for the invariability of the thermometer.

In studying these soils the results, in general, show that with the increase in concentration of ammonium sulfate solution the per cent of ad-

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sorption decreased, while the total amount of salt that went out of solution increased. Table I presents results very typical of the kind and well illustrates the above statements.

TABLE I[2]
ADSORPTION OF AMMONIUM SULFATE BY SASSAFRAS MEDIUM SANDY LOAM, USING 20 GM. OF SOIL AND 4 C.C. OF THE SOLUTION

Concentration of $(NH_4)_2SO_4$	Freezing-point Depression of the Solution in the Soil	Freezing-point Depression of the Original Salt Solution	Difference	Per cent Change in Depression
	°C.	°C.	°C.	
N/2	.850	1.063	.213	20.04
N/4	.408	.585	.177	30.26
N/8	.202	.315	.113	35.87
N/16	.093	.162	.069	42.60
N/32	.046	.087	.041	47.13
N/64	.023	.049	.026	53.06

The quartz sand, however, does not wholly follow the general rule, as do the agricultural soils. Instead of the decrease of the concentration of the salt solution on its addition to the soil, its concentration becomes greater, so far as we can judge by the freezing-point depression. This phenomenon is most pronounced in the coarse quartz sand and diminishes with the increase in the fineness of the material. Also, the effect is more noticeable in the more concentrated solutions than in those less concentrated. In fact, in the finer grades of the quartz treated with the comparatively dilute solutions, the point was reached after which the concentration of the resultant solution in the mixture with sand was

TABLE II[3]
ADSORPTION OF AMMONIUM SULFATE BY WASHED QUARTZ SAND

Quartz Sand, 24-mesh					Quartz Sand, 60-mesh				
Concentration of $(NH_4)_2SO_4$	Freezing-point Depression of the Solution in the Quartz Sand	Freezing-point Depression of the Original Solution	Difference	Per cent Change in Depression	Concentration of $(NH_4)_2SO_4$	Freezing-point Depression of the Solution in the Quartz Sand	Freezing-point Depression of the Original Solution	Difference	Per cent Change in Depression
	°C.	°C.	°C.			°C.	°C.	°C.	
N/4	.685	.586	—.099	—16.89	N/4	.704	.585	—.119	—20.35
N/8	.340	.297	—.043	—14.48	N/8	.349	.315	—.031	— 9.84
N/16	.177	.160	—.017	—10.63	N/16	.174	.162	—.012	— 7.41
N/32	.080	.086	—.006	— 6.98	N/32	.075	.087	.012	13.79
N/64	.047	.043	—.004	— 9.30	N/64	.034	.049	.015	30.61
					N/128	.011	.025	.014	56.00
					N/256	.006	.015	.009	60.00

[2] The figures in this table represent the determinations made in a single experiment, selected from several experiments of the same kind.

[3] The figures in this table represent the determinations made in a single experiment, selected from several experiments of the same kind.

less than that of the original solution. In other words, after a certain point, which evidently is specific for a given quartz sand, the quartz sand follows the same general rule that soils follow. To illustrate this point Table II may conveniently be cited.

Upon examining the figures in Table II, one notices that in the case of the coarse sand, which is designated as 24-mesh, in every instance the depression of the salt solution after application was greater than the depression of the freezing point of the solution before application. The per cent of the increase in depression, however, gradually decreased with the dilution of the applied solution. Turning to the results obtained with the finer grade of the quartz sand (60-mesh) one observes that in the first three concentrations there is a striking similarity to the results obtained with the coarsesr material. But, beginning with the concentration N/32, there is an adsorption of the salt by the quartz sand, the per cent of this adsorption increasing with the dilution of the solution.

The quartz sand used in these experiments was previously digested with the concentrated H_2SO_4 at room temperature for 15 hours and then washed with distilled water until no acid reaction was noticed on testing with litmus paper. This quartz sand when treated with distilled water usually gave a freezing-point depression of about .004 to .006° C. In view of these facts there could be no chemical reaction between the applied salt and the substances that would go into solution from the quartz sand itself, which would tend to increase the concentration of the resultant solution. It is not a case of negative adsorption, as observed by Gore (2), Williams (5), or Lagergren (3), and it is not similar to McCall's (4) selective adsorption, because these investigators dealt with systems of great internal surface area, such as charcoal suspension, or very finely ground soil. There, of course, was a possibility for a much greater adsorption of the water than of the ions of the salt tested. In the experiments here reported the phenomenon was most noticeable in the case where the internal surface area was least. To explain the observed behavior of the salt with the quartz sand the following hypothesis is here offered.

On the application of the ammonium sulfate to the quartz sand or the soil some of the ammonium ions are adsorbed more strongly than the sulfate ions. The excess of the sulfate ions remaining unite with 2H of the slightly ionized water, forming H_2SO_4. Sulfuric acid, as is well known, is more highly ionized than ammonium sulfate and, therefore, may cause a greater depression of the freezing point than the original solution of that salt. Owing to the small internal surface area, the adsorption of the coarse quartz sand is very small, but evidently is sufficient to change enough of the sulfate into sulfuric acid to show in the ionization of the resultant solution. On increasing the internal surface of the solid matter, the adsorption becomes greater, and, although more of $(NH_4)_2$ is adsorbed and more of H_2SO_4 is formed, some of the acid is also adsorbed, thus resulting in the decrease in the depression of the freezing

In the main article there will be presented some results of an experiment which will tend to prove the above contention. At this point is is sufficient to point out that the facts brought forth in Tables I and II have, to our mind, a considerable importance in pot cultures with soils and sands in both growing the agricultural plants and studying the development of microorganisms. In order to have comparable results it is of fundamental importance to know exactly the concentration of the soil solution after the application of the soluble salts to the soil or to the sands. In the former case it will always be less than the applied value, while in the latter case it may be less than, equal to, or greater than the value of the original solution before application, depending on the concentration of the salts applied and the fineness of the quartz used.

Besides these practical considerations the results seem to throw an additional light upon the cause of acidity in some of our agricultural soils. The hypothesis regarding the formation of acids in some soils due to the application of some commercial fertilizers finds an ample support in the results here presented.

In studying the other factors influencing the adsorption of ammonium sulfate in the soil, the results tend to show that the heavier the soil the greater is the amount of the salt adsorbed.

The time which the soil is allowed to be acted upon by the salt solution influences the per cent of the salt adsorbed. In the light sandy soil the maximum adsorption was reached in about 24 hours, while in the heavier type this point occurs after as many as 72 hours.

The temperature from 0° to 31° C. at which the reaction is allowed to proceed affects the degree of adsorption, this being greater at the higher temperature in a given time that at the lower one.

The presence of the organic matter in the form of dried blood, cottonseed meal, alfalfa, barley straw, or wheat straw, affects the adsorption of ammonium sulfate in the soil. Moreover, the application of these materials alone increases the concentration of the soil solution.

Literature Cited

(1) Bouyoucos, G. J., and McCool, M. M.
1915. The freezing-point method as a new means of measuring the concentration of the soil solution directly in the soil. Mich. Agr. Exp. Sta. Tech. Bul. 24, p. 10.

(2) Gore, G.
1894. Decomposition of liquids by contact with powdered silica, etc. *In* Proc. Birmingham Phil. Soc., v. 9, pt. 1; Chem. News, v. 69, p 22-46.

(3) Lagergreen, S.
1898. Zur Theorie der Sogenannten Adsorption Gelöster Stoffe. *In* Vet. Akad. Handl. Bihang K. Svenska, v. 24, II, no. 4, p. 3-39.

(4) McCall, A. G., Hildebrandt, F. M., and Johnston, E. S.
1916. The adsorption of potassium by the soil. *In* Jour. Phys. Chem., v. 20, no. 1, p. 51-63.

(5) Williams, A. M.
1914. On "negative" adsorption. *In* Trans. Farad. Soc., v. 10, pt. 1, p. 155-159.

IS THERE ANY FUNGUS FLORA OF THE SOIL?[1]

By

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INTRODUCTION

The earlier investigations on the microorganisms of the soil have revealed the fact that there exists a rather complex soil flora. By far the largest amount of work has been done on the bacteria of the soil, and only within recent years the study of the other groups of microorganisms has been taken up. Little attention has been paid to the fungi of the soil, and although several researches have been conducted with the purpose of isolating and identifying the soil fungi, and also studying their activities, such as cellulose decomposition and ammonification, the work was on the whole of a rather isolated character. No attempt was made to establish the fact, whether the fungi actually live in the soil or are mere occasional invaders; whether their presence in the soil is limited to certain areas or is wide-spread; whether the types present in one soil are also common in other soils, or are of a local character. While a great deal of information has been obtained on the points mentioned above for soil bacteria, very little is known about the other groups of microorganisms. It is a well known fact that certain groups of bacteria, such as the common ammonifying, nitrifying, nitrogen-fixing, are found commonly in the soil, if the environmental conditions are favorable. Is this true also for soil fungi? Do the species found in one soil occur also in another? Is their occurrence influenced by the type of soil, climate, fertilization, and other conditions which usually modify the soil flora?

If the fungi are not true soil organisms, if they are merely brought into the soil by some outside agencies, such as manure, one would naturally expect to find in one soil different forms from those in another soil. This investigation was undertaken with the purpose of ascertaining whether soils collected from different parts of the world, under entirely different climatic and other conditions—soils differently treated—would contain similar groups of organisms, or would vary according to the conditions.

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Since little work has been done on the identification of many groups of soil fungi, one would naturally expect that, while isolating these organisms from a number of soils, many unidentified species or even genera would be encountered. It was not the purpose of this investigation to describe new forms isolated from the soil, but only to attempt the determination of well identified forms, and so to compare the fungus flora of different soils on the basis of these identified species and genera. If forms were isolated which could not be identified with any degree of certainty, they were left out of consideration when found only in one or two soils. If such an organism was isolated from a number of soils, of if otherwise found important, it was mentioned as an unidentified form. No description of the organisms is given in this paper, since it was thought not advisable to give only a few meagre data, while a full description of all the forms studied was not possible for the present. A description of most of the forms mentioned in this paper is given in another place (18); some of the more important unidentified groups, such as Trichodermæ, Fusaria, Aspergilli, and Penicillia, were sent to investigators who make a special study of these groups of organisms. A number of forms, which are mentioned in this paper as unidentified, are under further study; the results will be published, if the data obtained are found to be of scientific importance.

Historical

A discussion of the literature on the occurrence and activities of soil fungi will be found in another place (18). Only those investigations will be mentioned here, which have a direct bearing upon the comparison of the fungi isolated from different soils. The investigations where only very few forms were isolated are omitted here.

In 1886 Adametz (1) isolated 11 fungi and 4 yeasts from a loamy and a sandy soil, at a depth of 25 to 30 cm., and did not find any difference in the fungus content of the two soils at the different depths. The forms isolated included *Penicillium glaucum, Mucor mucdo, Mucor racemosus, Aspergillus glaucus,* and *Oidium lactis.* Nikitinsky (13) isolated in 1902 from the soil *Penicillium glaucum, Aspergillus niger, Mucor mucedo,* and *Trichothecium* sp. Oudemans and Koning (14) isolated in 1902 45 species of fungi from a humus soil in Holland; a number of these organisms have later been found by other investigators in different parts of the world. Hagem (6) isolated the Mucorales from a number of soils in Norway, and came to the conclusion that the upper soil layers form a good medium for the growth of these organisms. Large number of Mucorales, Penicillia, Aspergilli, and Cladosporia were found in cultivated soils. He came to the conclusion that the Mucorales of cultivated soils differ very much from those of forest soils and consist of new species entirely. Namyslowski (11, 12) isolated from the soil in

1910 *Zygorhynchus Vuilleminii*, *Mucor microsporus*, and *Rhizopus arrhizus*. Beckwith (2) isolated in 1911 from "wheat-sick" soils of North Dakota the genera Fusarium, Colletatrichum, Macrosporium, Alternaria, Spicaria, Verticillium, Rhopolomyces, Cephalothecium, and Helminthosporium. Manns (5), several years before the work of Beckwith was published, isolated a number of fungi from "flax-sick" soils. These included representatives of the genera Fusarium, Penicillium, Aspergillus, Mucor, Alternaria, Hormodendrum, Cephalosporium, Cephalothecium, Diplocladium, Zygodesmus, Sporotrichum, Ovularea, and Briarea. The Fusaria were found most abundantly, especially in soils, where flax was grown continuously for a number of years. In 1912 Jensen (7) isolated from several soils of New York 35 species of fungi, and expressed his opinion that fungi parasitic on plants may live during the winter as soil saprophytes. Dale (4) isolated from sandy, chalky, peat and "black earth" soils of England over 100 organisms, many of which have also been isolated by the previous investigators. Goddard (5) isolated in 1913 from a rather rich clay loam Michigan soil 18 species of fungi, and did not find any variability as to their occurrence in different soil depths. He thought that there is a constant fungus flora in the soil, regardless of tillage and manuring.

McLean and Wilson (10) isolated from a New Jersey Sassafras loam 26 species of fungi. Traaen (17) isolated from Norway soils among others *Trichoderma Koningi*, *Trichoderma lignorum*, and *Mucor spinosus*. The author (18) has recently published an investigation on the soil fungi, isolated from 6 New Jersey soils, one soil from California and one from Oregon. Altogether over 100 species belonging to 31 genera were isolated. Werkenthin (20) isolated a number of fungi from Texas soils, and did not find any variation of fungi in regard to cultivated or virgin soil. He concluded therefore that there is a rather constant and characteristic fungus flora in the soil; Aspergilli seemed to be the predominant fungi in the South, while Penicillia and Mucorales are found more extensively in northern soils, and occur only occasionally in southern soils.

Experimental

Soils Used

A number of soils were collected from different parts of this country for the comparison of their fungus content. The samples were taken under sterile conditions at a depth of 1 to 8 inches, and shipped in sterile containers. As soon as the samples came to the laboratory, they were well mixed and plated out, care being taken to avoid all possible contaminations, by using sterile water and sterile dishes for the work. It may be stated here that all the samples, with two exceptions, came in good

order, the soil being moist, and the containers perfectly tight. Only the container with the Colorado soil was broken in transfer, but the sample for plating was taken from the central portion, so as to avoid as much as possible the outside part of the sample; the North Dakota soils were almost air-dried, but since the containers were closed, there was probably little contamination, although some of the organisms might have died.

In all, 25 soils were studied. More work has been done on the first four New Jersey soils, because these were situated at a short distance from the laboratory; therefore more forms are reported from these soils than from the others, since more samples were taken at different depths and in different seasons of the year. The complete results of the fungi of these four soils have been reported before (18), but some of the data will be repeated here so as to have them available for comparison.

The soils will be designated in the following manner:

1. *New Jersey garden soil,* a Sassafrass sandy loam, used by the botanical department of the New Jersey Agricultural Experiment Station, manured every year for the last 20 years with 20 tons per acre of stable manure annually, and receiving an application of lime every 5 years. This soil has an almost neutral reaction, as determined by the Veitch method.

2. *New Jersey orchard soil,* a Sassafrass sandy loam, near the garden soil, of exactly the same texture and structure. This soil is the unfertilized plot of an apple orchard receiving no application of manure or fertilizer for the last 20 years; only a cover crop of oats has been grown for the last 3 years; the soil receives 8 to 10 cultivations through the summer, and is of a rather acid character.

3. *New Jersey meadow soil,* an Alloway clay, under grass for the last 6 years. This is a heavy soil, with a high moisture, nitrogen, and organic matter content.

4. *New Jersey forest soil,* of the same type as the first two soils, but not cultivated for the last 50 years, if ever. It contains a large amount of undecomposed organic matter, and is of a pronounced acid character.

5. *New Jersey Sassafrass gravelly loam,* a medium loamy soil, under irregular rotation of crops.

6. *New Jersey iron soil,* a medium loam termed "Colts Neck loam," containing 48 per cent of Fe_2O_3, which gives to the soil a brick-red appearance; this soil is situated near Keyport, N. J., and is in a peach orchard.

7. *New Jersey muck land,* from Bergen County, N. J. This soil is rich in organic matter and is heavily manured annually; celery has been grown on this land continuously for over 9 years.

8. *Milltown cranberry soil.* This sample was taken from a soil, on which wild cranberries were grown, near Milltown, N. J. It is possible that the bog was once cultivated, although no information could be ob-

tained on this point. The soil was soaked with water at the time of sampling, and proved to be very acid, having a lime requirement, by the Veitch method, of nearly 8000 pounds of CaO per acre.

9. *Buckalew cranberry bogs*, near Jamesburg, N. J. A number of samples were taken from these bogs, which were covered with water at the time of sampling, to a depth of 6 to 24 inches. The fungi isolated from these samples will be reported together, although some differences were observed between the different samples, depending on the soil type, method of fertilization and treatment.

10. *Iowa Carrington loam*, humus plot of the Iowa Agricultural Experiment Station at Ames; the soil was clean cultivated for the last 7 years and received annually an application of manure.

11. *Louisiana sandy loam*, on which sugar cane was grown for many years, two or three years in succession, with corn as a rotation crop; the fertilizer applied to this soil for the last 10 years consisted of acid phosphate and tankage.

12. *California fertilized soil* at Riverside, on which naval oranges were grown. This soil received stable manure and rock phosphate; vetch was grown as a winter cover crop; the soil was irrigated and frequently cultivated.

13. *California unfertilized soil* at Riverside, with the same system of cropping, cultivation, and irrigation, as the previous soil, but receiving no fertilizer.

14. *Oregon black muck soil*, from the Willamette valley, of a high organic matter content, which benefited greatly by drainage and liming.

15. *Oregon white land*, from the Willamette valley, not very productive and benefitted by drainage.

16. *Porto Rico soil*, a heavy clay loam, from the vegetable garden of the experiment station. This soil had an application of barnyard manure, tobacco stems, sodium nitrate, and acid phosphate at frequent intervals during the last 5 years; truck crops were grown on it for the last 3 years.

17. *North Dakota wheat soil*, from the experiment station plot.

18. *North Dakota flax soil*, from a constant flax bed, inoculation area.

19. *Honolulu pineapple soil*, taken from between the rows of pineapples of the Hawaiian station plots. The soil had recently a rather liberal growth of Sunn hemp (*Crotalario juricea*), worked under as green manure.

20. *Sitka garden soil*, from Sitka, Alaska. This sample was taken from the improved land, used for horticultural experiments.

21. *Texas soil*, a Lufkin fine sandy loam, from the college garden, which has been under cultivation for the last 15 years; as many as six crops per season have been grown on it. The year previous to sampling it received 200 pounds of acid phosphate per acre, the soil is diseased to

tomatoes, squashes, and cucumbers, the roots of the plants being usually attacked.

22. *Colorado soil,* at Fort Collins, under alfalfa for the last 10 years.

23. *Maine Aroostook soil,* from Presque Isle, Maine, the best potato soil in the locality.

24. *Maine Aroostook infested soil,* from Presque Isle, infested with scale.

25. *Alberta soil,* from Edmonton South, Canada; the land was in bush till 1909, then cleared; two crops of oats and three of grass have been grown since, when the samples were taken, the plots were used for demonstration purposes.

Media Used

The media used were the same as those described in the previous paper, namely:

1. *Modified Albumen Agar,* composed as follows:

Distilled water	1000 c.c.
Dextrose	10.00 gm.
K_2HPO_4	0.50 gm.
$MgSO_4$	0.20 gm.
Egg-albumen	0.15 gm.
$Fe_2(SO_4)_3$	Trace
Agar	15.00 gm.

This medium was used for the isolation of the organisms from the soil.

2. *Raisin Agar*: raisin extract, to which 25 gm. of agar per litre were added; this medium was used for the purification of the fungi from bacteria.

3. *Cook's No. II medium*:

Distilled water	1000 c.c.
Dextrose	20.00 gm.
Peptone	10.00 gm.
K_2HPO_4	0.25 gm.
$MgSO_4$	0.25 gm.
Agar	15.00 gm.

This medium was used for the study of the Mucorales.

4. *Czapek's Synthetic Solution Agar*:

Distilled water	1000 c.c.
$MgSO_4$	0.50 gm.
K_2HPO_4	1.00 gm.
KCl	0.50 gm.
$FeSO_4$	0.01 gm.
$NaNO_3$	2.00 gm.
Cane sugar	30.00 gm.
Agar	15.00 gm.

This medium was used for the study of all the organisms, except the Mucorales.

In the process of isolation of fungi from the soil two methods of procedure have been followed:

1. *The dilution method* (18). When the soil samples came to the laboratory, they were at once mixed and a weighed portion was shaken with sterile water. Plates of 20,000 and of 200,000 dilution were poured, albumen agar being used as the medium for growth. The plates were then incubated at 22° to 25° C. for 6 to 10 days, the period of incubation being uniform for all the soils, then the bacteria and the fungi were counted. The latter were transferred with a sterile platinum loop to sterile slants of Czapek's agar.

2. *The direct inoculation method* (19), by which lumps of soil were placed in the center of a sterile Petri dish containing cooled Czapek's agar. The plates were allowed to incubate for 24 hours at 22° C. At the end of that period, the mycelium that had grown out of the lump into the medium was transferred with a sterile platinum needle to slants of Czapek's agar for further study.

The cultures were allowed to grow on the slant until spore-production took place. Single spore cultures were then made, raisin agar being used as a medium for transfer, so as to eliminate any bacterial contamination.

Numbers of Fungi in the Soil

The numbers of fungi have been determined in all the soils. These were calculated on the basis of air-dried soil.

The counts were made from the plates, modified albumen agar being used; the plates were poured in triplicate. The numbers of bacteria and of actinomyces varied slightly on the different plates, but the numbers of fungi varied greatly, one plate often containing twice as many fungi as the other plate (Table I).

There does not seem to be any relationship between the numbers of fungi obtained by this method and the locality from which the soil was taken, method of cultivation, treatment, etc. Although the fact could be pointed out that where the numbers of bacteria and actinomyces were high, the numbers of fungi obtained by the dilution method also were high, they were subject to much greater variations than the numbers of bacteria and actinomyces. In general, this method of obtaining an insight into the fungus content of the soil is subjected to a great deal of criticism, since it does not give us a true idea of the fungi of the soil. It is by far not so satisfactory as the counting of bacteria and actinomyces developing on plates made from high dilutions. For this reason, little significance should be attached to the counts of fungus numbers in the soil. This is made clear by comparing the fungi obtained by the dilution and actual isolation methods. Soils, which would indicate much higher numbers of fungi than others by the dilution method, would give, on the

actual isolation of the mycelium, not more and often even smaller numbers of organisms than the others. Of course, it might be argued that in the dilution method we obtain not only the pieces of mycelium, but also the numbers of fungus spores in the soil; if the spores are counted, one might find in one spot a large number of them and in another only very few. This is actually proven by the plates: not only do the duplicates check very poorly, so that on one plate two or more times as many colonies may be obtained as on its duplicate, but the different dilutions will give entirely different results; for example, a 1000 dilution may give 15 colonies, a 10,000—8 colonies, and a 100,000—4 or 5 colonies.

The data are presented in Table I not for any great value that should be attached to them, but merely to show the variability of the numbers of fungi obtained from different soils, when these were treated alike, and also the lack of relation between the numbers of fungi obtained by the dilution method and soil characteristics.

TABLE I
NUMBER OF MICROORGANISMS PER GRAM OF AIR-DRIED SOIL

Soil Used	Bacteria	Actinomyces	Fungi
New Jersey garden	7,202,000	711,000	313,000
New Jersey orchard	8,257,000	611,000	375,000
New Jersey meadow	10,133,000	900,000	925,000
New Jersey forest	2,088,000	20,000	218,000
New Jersey muck	2,600,000	.....	150,000
Milltown bogs	185,000	.....	33,000
Buckalew bogs	450,000	12,000	43,000
Iowa soil	2,200,000	281,000	113,000
Louisiana soil	10,000,000	2,000,000	119,000
California fertilized	3,840,000	680,000	108,000
California unfertilized	644,000	356,000	36,000
Oregon muck	7,900,000	1,400,000	400,000
Oregon white land	3,400,000	300,000	300,000
Porto Rico soil	2,140,000	960,000	300,000
North Dakota wheat	2,070,000	933,000	30,000
North Dakota flax	1,730,000	263,000	23,000
Hawaiian soil	4,335,000	665,000	76,000
Alaska soil	6,035,000	1,566,000	330,000
Texas soil	2,125,000	574,000	30,000
Colorado soil	2,440,000	1,560,000	230,000
Maine Aroostook loam	4,650,000	250,000	85,000
Maine Aroostook infested	15,900,000	2,200,000	300,000
Canada soil	1,600,000	1,100,000	112,000

When we come to study the types of fungi isolated from the different soils, an entirely different relationship is found. By a proper examination of the fungus flora of different soils, one will be at once struck by the presence of common types in many soils and by the peculiarity of the soil flora, depending on the soil type, its organic content, fertilization method of cropping, acidity, climatic and other environmental conditions.

In Table II are given the types of fungi isolated from 24 different soils of North America and one from the Hawaiian Islands. In many cases two or more samples were obtained, but if the differences in the fungi found were not sufficient to differentiate between two soils of the same locality, they were classed together.

The failure to obtain any species from any particular soil should not be taken as an indication that the organism was absolutely lacking in that soil, but that it was not found in the sample taken. Had the number of samples taken from any one soil been large enough, many other species might have been obtained. More species have been isolated from the first four New Jersey soils, because a number of isolations have been made from each. But even one isolation is sufficient, if only all the possible forms have been transferred from the plates and record kept of the frequency of each, to give us an idea as to the character of the fungus flora of the particular soil. Care should then be taken to use for isolation purposes several media, so as to bring out as many groups of soil fungi as possible.

Looking through Table II one might observe some very important differences in the groups of fungi occurring in the soil, depending on the soil conditions. It is seen that the most widely spread groups of soil fungi are the Mucors, Aspergilli, Penicillia, Trichodermæ, and Rhizopuses; Cephalosporia, Fusaria and other groups following. But the occurrence of these groups in all the different soils is not constant and characteristic to all the soils. It looks as if the cooler northern soils contain an abundance of Mucors, Trichadermæ, Fusaria, Penicillia, and others; while the Aspergilli form the more common group of organisms in the southern soils. This has already been noted by Werkenthin (20).

Taxonomic Consideration of the Fungi Found in the Soil

The arrangement of the description of the Hyphomycetes has been made according to the classification adopted by Lindau in Rabenhorst's Kryptogamen Flora (8). The same sources have been used for the identification of the fungi isolated from the different soils as those mentioned in the previous paper (18); particular use has been made of the work of Hagem (6) in the indentification of the Mucors, that of Chivers (3) for the Chætomia, the work of Thom (16) and Westling (21) for the Penicillia, and that of Sherbakoff (15) for the Fusaria. The additional Chætomia have been identified again by Dr. A. H. Chivers, of Dartmouth College, and several Hyphomycetes by Dr. R. Thaxter, of Harvard University, to whom the author is greatly indebted for their kindness.

Phycomycetes

Mucorales

The most common representatives of this group of organisms in the soil were found to be *Mucor racemosus, Mucor circinelloides, Rhizopus nigricans, Zygorhynchus Vuilleminii, Mucor hiemalis,* and *Rhizopus nodosus.*

Mucor racemasus Fres. was isolated by the author from 17 soils of North America and from the Hawaiian soil. It has also been isolated

TABLE II

LIST OF SPECIES OF FUNGI ISOLATED FROM THE DIFFERENT SOILS

Name of Organism	New Jersey Garden	New Jersey Orchard	New Jersey Meadow	New Jersey Forest	New Jersey Iron	New Jersey Sassafras Loam	New Jersey Muck	Milltown Boxs	Buckalew Boxs	Iowa Soil	Louisiana Soil	California Fertilized	California Unfertilized	Oregon Muck	Oregon White Land	Porto Rico Soil	North Dakota Wheat	North Dakota Flax	Hawaiian Soil	Alaska Soil	Texas Soil	Colorado Soil	Maine Aroostook Loam	Maine Aroostook Infested Soil	Canada Soil
Absidia Lichteimi (Lucet et Const.) Lendner	*	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	*	*	:
Absidia Orchidis (Vuill.) Namys.	:	:	:	:	*	:	:	:	:	:	:	:	:	:	:	:	:	:	*	:	:	:	:	:	:
Absidia cylindrospora Hagem.	:	*	:	*	:	*	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	*	:	:
Mucor hiemalis Wehmer	:	*	*	:	:	*	*	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	*	:	:
Mucor microsporus Namys.	:	*	*	*	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Mucor circinelloides van Tieghem	:	:	*	:	:	*	:	*	:	:	:	:	:	*	:	:	*	:	:	:	:	:	*	*	:
Mucor plumbeus Bon.	:	*	*	*	:	:	*	:	*	:	:	:	:	:	:	:	:	:	*	:	:	:	:	:	:
Mucor racemosus Fres.	:	*	*	:	:	:	*	:	:	:	*	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Mucor silvaticus (?) Hagem.	:	:	:	:	:	*	:	*	:	:	:	:	:	:	:	:	:	:	:	*	:	:	:	:	:
Mucor botryoides Lendner	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Mucor saturninus (?) Hagem.	:	*	*	:	:	:	:	*	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Mucor spharosporus Hagem.	:	:	*	*	*	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Mucor glomerula (Banier) Lendner	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Mucor Ramannianus Möller	:	*	*	*	*	:	:	*	*	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Mucor flavus Banier	:	*	*	*	:	:	*	*	*	:	:	*	:	:	*	:	:	:	:	:	:	:	:	:	:
Zygorhynchus Vuilleminii Namys.	:	*	*	:	:	:	:	*	:	:	*	:	:	:	:	*	:	:	:	:	:	:	:	:	:
Rhizopus nigricans Ehren.	:	:	*	:	:	:	:	*	:	:	:	:	:	:	*	:	:	:	:	:	:	:	:	:	:
Rhizopus nodosus Namys.	:	:	*	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Chætomium olivaceum Cooke & Ellis	:	:	:	:	:	*	:	:	*	:	:	:	*	:	:	:	:	:	*	:	:	:	:	:	:
Chætomium cochliodes Palliser	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Chætomium globosum	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	*	:	:
Chætomium funiculum	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Hypoderma sp.	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Sordaria sp.	:	:	:	:	:	*	:	:	*	:	:	:	:	:	:	:	:	*	:	:	:	:	:	:	:
Spharonema sp.	*	:	*	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Monilia sitophila (Mont.) Sacc.	:	:	*	:	:	:	:	:	*	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Monilia humicola (?) Oud.	:	*	*	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Oidium lactis Fres.	*	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:

TABLE II—(Continued)

LIST OF SPECIES OF FUNGI ISOLATED FROM THE DIFFERENT SOILS

Name of Organism	New Jersey Garden	New Jersey Orchard	New Jersey Meadow	New Jersey Forest	New Jersey Iron	New Jersey Sassafras Loam	New Jersey Muck	Milltown Bogs	Rucklelew Bogs	Iowa Soil	Louisiana Soil	California Fertilized	California Unfertilized	Oregon Muck	Oregon White Land	Porto Rico Soil	North Dakota Wheat	North Dakota Flax	Hawaiian Soil	Alaska Soil	Texas Soil	Colorado Soil	Maine Aroostook Loam	Maine Aroostook Infested Soil	Canada Soil
Cephalosporium acremonium Corda	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Cephalosporium Koningi Oud	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Cephalosporium curtipes (?) Sacc	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Cephalosporium sp. (G. 23)	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Cephalosporium sp. (D. 32)	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Cephalosporium sp. (C. 56)	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Papulospora pannosa	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Trichoderma Koningi Oud	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Trichoderma lignorum (Tode) Hartz	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Trichoderma album Preuss	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Trichoderma sp. (yellow)	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Trichoderma sp. (G. 5)	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Trichoderma sp. (C. 10)	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Trichoderma sp. (D. 34)	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Trichoderma sp. (J. 6)	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Aspergillus fumigatus Fres	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Aspergillus nidulans Eidam	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Aspergillus diversicolor Vuill	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Aspergillus niger van Tieghem	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Aspergillus sp. (C. 19)	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Aspergillus glaucus Link	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Aspergillus calyptratus (?) Oud	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Aspergillus fuscus Schiemann	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Aspergillus flavus Link	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Aspergillus repens de Bary	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Aspergillus clavatus Desmasières	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Aspergillus globosus Jensen	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Aspergillus sp. (R. 11)	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..

TABLE II—(Continued)

LIST OF SPECIES OF FUNGI ISOLATED FROM THE DIFFERENT SOILS

Name of Organism	New Jersey Garden	New Jersey Orchard	New Jersey Meadow	New Jersey Forest	New Jersey Iron	New Jersey Sassafras Loam	New Jersey Muck	Millstown Bogs	Buckshaw Bogs	Iowa Soil	Louisiana Soil	California Fertilized	California Unfertilized	Oregon Muck	Oregon White Land	Porto Rico Soil	North Dakota Wheat	North Dakota Flax	Hawaiian Soil	Alaska Soil	Texas Soil	Colorado Soil	Maine Aroostook Loam	Maine Aroostook Infested Soil	Canada Soil
Aspergillus sp (M. 23)	*										*		*			*									
Penicillium luteum Zukal	*		*		*		*	*		*	*														
Penicillium luteum group Thom	*		*						*																
Penicillium lilacinum Thom			*	*																					
Penicillium pinophilum (Hedgcock) Thom				*			*		*		*					*						*			
Penicillium chrysogenum Thom			*			*										*		*							
Penicillium purpurogenum var. *rubri sclerotium* Thom																									
Penicillium commune Thom		*												*	*										
Penicillium decumbens Thom				*	*		*		*								*								
Penicillium digitatum Sacc				*		*		*				*	*												
Penicillium expansum (Link) Thom														*											
Penicillium italicum Wehmer				*	*							*													
Penicillium axalicum Thom				*																					
Penicillium notatum Westling				*																					
Penicillium viridicatum Westling			*		*																				
Penicillium atramentosum Thom		*																							
Penicillium glabri Wehmer				*	*			*																	
Penicillium Pfefferianum[1] Wehmer										*															
Penicillium sp.[1] (H)				*				*																	
Penicillium rugulosum Thom									*																
Penicillium lividum Westling						*																			
Penicillium cyclopium Westling																									
Penicillium roqueforti Thom																									
Penicillium desiccens Oud			*				*		*				*			*									
Penicillium intricatum Thom																									
Penicillium frequentes Westling						*																			

[1] Organisms belonging to Wehmer's genus Citromyces.

TABLE II—(Continued)

LIST OF SPECIES OF FUNGI ISOLATED FROM THE DIFFERENT SOILS

Name of Organism	New Jersey Garden	New Jersey Orchard	New Jersey Meadow	New Jersey Forest	New Jersey Iron	New Jersey Sassafras Loam	New Jersey Muck	Millstown Hogs	Buckshaw Hogs	Iowa Soil	Louisiana Soil	California Fertilized	California Unfertilized	Oregon Muck	Oregon White Land	Porto Rico Soil	North Dakota Wheat	North Dakota Flax	Hawaiian Soil	Alaska Soil	Texas Soil	Colorado Soil	Maine Aroostook Loam	Maine Aroostook Infested Soil	Canada Soil
Penicillium sp. (Q. 44)							*																	*	
Penicillium sp. (N. 39)																									
Penicillium sp. (D. 35)																									
Penicillium group I (13-25)																									
Penicillium group II (6-22-23)																									
Penicillium group III (9-24)																									
Penicillium group IV (2-11-15)																									
Penicillium group V (18-20)																									
Penicillium group VI (10)																									
Penicillium sp. (n. var?)																									
Scopulariopsis brevicaule Sacc.																									
Sporotrichum sp. I																									
Sporotrichum roseum (?) Link																									
Sporotrichum sp. III																									
Sporotrichum sp. IV																									
Rhinotrichum sp.																									
Sepedonium chrysospermum Bull.																									
Botrytis cinerea Pers.																									
Verticillium glaucum (?) Bonorden																									
Verticillium terrestre (?) (Link) Lindau																									
Verticillium sp.																									
Verticillium albus Preuss. var. *nana* Oud.																									
Acrostalagmus cinnabarinus																									
Aleuriosporium roseum Corda																									
Cephalothecium roseum Corda																									
Zygodesmus sp.																									
Stachybotrys alternans Bonorden																									
Zygodesmus sp.																									
Acremoniella fusca var. *minor* Corda																									
Acremoniella sp. (?) (C. 37)																									

TABLE II—(Continued)

LIST OF SPECIES OF FUNGI ISOLATED FROM THE DIFFERENT SOILS

Name of Organism	New Jersey Garden	New Jersey Orchard	New Jersey Meadow	New Jersey Forest	New Jersey Iron	New Jersey Sassafras Loam	New Jersey Muck	Milltown Bogs	Bucklew Bogs	Iowa Soil	Louisiana Soil	California Fertilized	California Unfertilized	Oregon Muck	Oregon White Land	Porto Rico Soil	North Dakota Wheat	North Dakota Flax	Hawaiian Soil	Alaska Soil	Texas Soil	Colorado Soil	Maine Aroostook Loam	Maine Aroostook Infested Soil	Canada Soil
Dematium pullulans (?) de Bary	:	:	:	:	:	:	:	:	:	:	:	*	:	:	:	*	:	:	:	:	:	:	:	*	:
Hormodendrum Hordei Bruhne	:	*	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	*	:	*	:	:	:
Dicoccum asperum Corda	:	:	:	:	*	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Bactisporium gallarum (?) Molliard	:	*	*	*	:	*	:	:	:	:	:	:	:	:	:	:	*	*	:	*	*	*	:	:	:
Cladosporium herbarum Pers	:	:	:	:	:	:	:	*	:	*	*	*	:	:	*	:	:	:	:	:	:	:	:	:	:
Cladosporium epiphyllum Pers	:	*	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Cladosporium sp.	*	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	*	:	:	:	:	:
Trichocladium asperum Hartz	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	*	:	:	:	:	:	:	:	:
Alternaria humicola (?) Oud	:	:	*	:	:	*	*	:	*	:	:	:	:	:	:	:	:	*	:	:	:	*	:	:	:
Alternaria sp. (B. 20)	:	*	:	:	:	:	:	:	:	*	:	:	:	:	:	:	*	:	:	:	:	:	:	:	:
Alternaria sp. (A. 36)	*	:	*	:	:	*	:	:	*	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Macrosporium sp.	:	:	:	:	:	:	:	:	:	:	*	:	:	:	:	:	*	:	:	:	:	*	:	:	:
Helminthosporium sp.	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	*	:	:	:	:	:	:	:	:
Myrothecium roridum Tode	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Stysanus stemonites (Pers.) Corda	:	:	:	:	:	*	:	:	:	:	:	*	:	:	:	:	:	:	:	:	*	:	:	*	:
Fusarium angustum Sherb	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Fusarium bulbigenum Sherb	:	:	:	:	:	:	:	:	:	:	*	*	:	:	:	:	:	:	:	*	*	:	:	:	:
Fusarium solani Ap. et Wr	:	:	:	:	:	:	:	:	*	:	*	*	:	:	:	:	:	:	:	:	:	:	:	:	:
Fusarium orthoceras Ap. et Wr	:	*	:	:	:	:	:	:	:	:	:	:	*	:	:	*	:	:	:	:	:	:	:	*	:
Fusarium oxysporium var. *resupinatum* Sherb	:	:	:	*	*	:	:	:	:	*	:	*	:	:	:	:	:	:	:	:	:	:	:	:	:
Fusarium caudatum Wr	:	:	:	:	:	:	*	:	:	:	:	:	:	:	:	:	:	*	:	:	*	*	:	:	:
Fusarium oxysporium Schlech	:	:	:	*	*	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Fusarium lini (?) Bolley	:	:	*	:	:	:	:	*	:	:	*	:	:	:	:	*	*	*	:	:	:	:	:	:	:
Fusarium sp. (C.)	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Fusarium sp. (H. 28)	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	*	:
Melanconium sp. (G. 20)	*	:	:	:	*	*	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:
Coniothecium Fuckelii (?) Sacc	:	:	*	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:	:

by Adametz from sandy and loamy arable soils in Germany, Lendner in Switzerland, Hagem in Norway, Dale in England, Jensen in New York, McLean and Wilson in New Jersey and Werkenthin in Texas. This organism is of wide occurrence, having been isolated repeatedly by different investigators from soils in different parts of the world.

Mucor circinelloides Van Tieghem was isolated by the author from 12 different soils of North America; also by Hagem in Norway, Dale in England, and Jensen in New York.

Rhizopus nigricans Ehrenberg was isolated from 11 soils of North America and the Hawaiian soil; also by Adametz in Germany, Hagem in Norway, Jensen in New York, McLean and Wilson in New Jersey, and Werkenthin in Texas.

Zygorhynchus Vuilleminii Namys. was isolated from 12 soils of North America and was found one of the most common and most widely spread soil fungi; also by Namyslowski in Austria, Hagem in Norway, Jensen in New York, and McLean and Wilson in New Jersey.

Mucor hiemalis Wehmer was isolated by the author from 6 different soils of North America; also by Hagem in Norway, Lendner in Switzerland, Namyslowski in Austria, Jensen in New York, and McLean and Wilson in New Jersey.

Rhizopus nodosus Namys. was isolated by the author from 6 different soils; also by Lindner in Switzerland and Hagem in Norway.

The other Mucorales have been found each in a limited number of soils and do not seem to be of such wide occurrence as the first six organisms, although some of them were also isolated by other investigators in Europe and in this country. A number of other Mucorales were obtained, not given in Table II, but since they could not be identified with any great certainty and due to their limited occurrence, they have not been mentioned here.

Ascomycetes

A number of species of the genus Chætomium have been isolated from the different soils: *Chætomium globosum* from the New Jersey Sassafrass loam and Buckalew bogs; *Chætomium fumicolum* from the California unfertilized soil and Maine Aroostook loam; *Chætomium cochliodes* Palliser from the New Jersey Sassafrass loam, California unfertilized, and Hawaiian soils; *Chætomium olivaceum* Cooke and Ellis from the New Jersey meadow soil, also by Jensen in New York.

Hypodema sp. was isolated from the Hawaiian soil; *Sordaria* sp. from Texas soil; *Sphæronema* sp. from the Buckalew bogs.

A number of Aspergilli and Penicillia producing perfect stages were isolated, but these will be considered under the Hyphomycetes.

Fungi Imperfecti

Hyphomycetes

Mucedenaceæ

The largest number of species of soil fungi belong to one of the divisions of the Fungi Imperfecti, namely the Hyphomycetes, and the majority of them are included in the family Mucedenaceæ.

Several species of Monilia were isolated from the different soils, but only two of them could be identified, namely: *Monilia sitophila* (Mont.) Saccardo, isolated from the New Jersey garden, meadow, and sassafrass soils; *Monilia humicola* (?) Oud. isolated from the New Jersey meadow soil and Buckalew bogs, also by Köning in Holland.

Several species of Oidia were isolated, but only *Oidium lactis* Fres. was identified; it was isolated from New Jersey garden, orchard, and meadow soils, also by Adametz in Germany.

Sepedonium chrysospermum Bull. was isolated only once from the New Jersey muck soil. *Rhinotrichum* sp. was isolated once from the New Jersey meadow soil.

Of the important group of soil Cephalasporia a number of species were isolated, but, due to the difficulty of identification, only several of them are mentioned here, particularly those that were isolated from several soils or those that could be identified with a greater or less degree of certainty. *Cephalosporium Koningi* (?) Oud. was isolated once from Alaska soil, also by Köning in Holland; *Cephalosporium acremonium* (?) Cord. from New Jersey meadow soil, also by Köning in Holland; *Cephalosporium curtipes* (?) Sacc. from New Jersey meadow, Porto Rico, and Colorado soils; *Cephalosporium* sp. (g. 23) from New Jersey iron soil; *Cephalosporium* sp. (D. 32) from New Jersey forest, Oregon muck, Oregon white land, Porto Rico, North Dakota wheat, North Dakota flax, Hawaiian, and Maine Aroostook infested soils; *Cephalosporium* sp. (C. 56) from New Jersey meadow, California unfertilized, Oregon muck, and North Dakota flax soils.

Papulospora pannosa, identified by Dr. R. Thaxter, was isolated from Porto Rico, California fertilized, and California unfertilized soils.

The Trichodermæ, which have been divided in the previous paper (18) into 5 different strains, were found to be widely spread in most of the soils examined, particularly in those of an acid character and with a plentiful supply of moisture. They were found to live extensively in these soils, since not only the dilution method, but also the direct isolation method has revealed their presence. The species that agreed more closely with *Trichoderma Koningi* Oud. than any other one, was isolated from New Jersey meadow, forest, iron, and Sassfrass loam soils, Milltown and Buckalew bogs, Iowa, Louisiana, California fertilized, California unfertilized, Oregon white land, Porto Rico, Hawaiian, Alaska,

Maine Aroostook loam, and Maine Aroostook infested soils; also by Köning in Holland, Jensen in New York, Traaen in Norway, Dale in England, and McLean and Wilson in New Jersey.

The species which was thought to be *Trichoderma lignorum* (Tode) Harz was isolated from New Jersey meadow and forest soils, Milltown and Buckalew bogs, Louisiana, Oregon muck, Porto Rico, Maine Aroostook infested, and Canada soils; also by Jensen in New York. *Trichoderma album* Preuss. was isolated from New Jersey forest and Louisiana soils, also from the Buckalew bogs. *Trichoderma* sp. (g. 5) was isolated from New Jersey garden, forest, iron and muck soils, Milltown and Buckalew bogs, Iowa, Louisiana, California fertilized, California unfertilized, Hawaiian, and Alaska soils. *Trichoderma* sp. (C. 10) was isolated from New Jersey meadow and forest soils, also from Porto Rico, North Dakota wheat, Hawaiian, Maine Aroostook loam, and Maine Aroostook infested soils; *Trichoderma* sp. (D. 34) from New Jersey forest, Hawaiian, and Maine Aroostook loam soils; *Trichoderma* sp. (J. 6) from New Jersey muck, Milltown and Buckalew bogs, Louisiana, and Maine Aroostook loam soils. A yellow species of *Trichoderma* was isolated from Texas and Oregon soils.

In the next two genera, Aspergillus and Penicillium, we find two of the most representative groups of soil organisms, which, with the exception of the Mucorales in some soils and Trichodermæ in others, lead, both in the total number and in the number of species isolated, the groups of soil fungi. About 25 species of Aspergilli and 50 species of Penicillia were isolated from the different soils. Not all of them could be identified, a good many of them are probably new to science; the identification of these as well as of the other groups of fungi will be left to the special student; only mention will be made here of those organisms, which were identfied, or of those that were isolated several times from the soil.

Aspergillus fumigatus Fres. was isolated a number of times from the New Jersey garden, orchard, meadow, forest, and Sassafrass loam soils, Milltown bog, Iowa, North Dakota wheat, North Dakota flax, and Texas soils; also by Jensen in New York, and Werkenthin in Texas; *Aspergillus nidulans* Eidam, from New Jersey garden, orchard, meadow, forest, Buckalew bogs, and Texas soils; also by Goddard in Michigan; *Aspergillus diversicolor* Vuill., from New Jersey garden, orchard, meadow, forest, iron, and California fertilized soils; *Aspergillus niger* Van Tieghem, from New Jersey garden, orchard, meadow, Sassafrass loam, Louisiana, California fertilized, California unfertilized, Porto Rico, and Texas soils; also by Nikitinsky in Germany, Dale in England, and Werkenthin in Texas; *Aspergillus flavus* Link, from New Jersey Sassafrass loam, Louisana, California fertilized, California unfertilized, Porto Rico, Hawaiian, and Texas soils; *Aspergillus clavatus* Desmasières, from New Jersey Sassafrass loam, New Jersey muck, Buckalew

garden, meadow, and muck soils, Buckalew bogs, North Dakota wheat, North Dakota flax, Alaska, and Texas soils.

A number of Verticillia were isolated from the different soils, but none of them was identified with certainty.

Cephalothecium roseum Corda was isolated from New Jersey meadow, Porto Rico, North Dakota wheat and flax soils; also by Jensen in New York.

Zygodesmus sp. was isolated from New Jersey forest, Louisiana, and Oregon muck soils. *Acrothecium* sp. was isolated from Porto Rico soil; and *Stachobotrys alternans* Bon., from Porto Rico soil. *Acremoniella fusca,* var. *minor* Corda was isolated from Iowa, North Dakota wheat, and Alaska soils; *Acremoniella* sp. (C. 37), from New Jersey meadow soil.

Dematiaceæ

Basisporium gallarum Molliard was isolated from New Jersey iron soil; *Hormodendrum Hordei* Bruhne, from Alaska soil; also by Jensen in New York; *Cladosporium herbarum* (Pers.) Link, from New Jersey garden, orchard, meadow, forest, sassafrass loam, Milltown bogs, Louisiana, California fertilized, Oregon white land, and Texas soils; also by Jensen in New York, Dale in England, and Goddard in Michigan; *Cladosporium epiphyllum* Pers., from New Jersey garden and meadow soils, California fertilized and Texas soils. *Trichocladium asperum* Harz was isolated from Canada soil; *Dematium pollulans* de Bary, from California fertilized, Porto Rico, and Aroostook infested soils. A number of Alternaria have been isolated from the different soils, but none of them could be identified with any degree of certainty. *Helminthosporium* sp. was isolated from North Dakota wheat, Colorado, and Canada soils. *Myrothecium roridum* Tode, identified by Dr. R. Thaxter, was isolated from North Dakota wheat soil; and *Macrosporium* sp., from New Jersey Sassafras loam and Louisiana soils.

Tuberculariaceæ

A comparatively large number of species of Fusaria have been isolated, but also a great many of these have been left unidentified. *Fusarium angustum* Sherb. was isolated from New Jersey Sassafras loam. *Fusarium bullatum* Sherb. was isolated from Louisiana, Alaska, Canada, and one New Jersey soil. *Fusarium solani* (Mart.) Ap. et Wr., from Louisiana, California fertilized and unfertilized, Hawaiian, Texas, Colorado, Maine Aroostook infested, and one New Jersey soil; also by Dale in England and Werkenthin in Texas. *Fusarium orthoceras* Ap. et Wr., from New Jersey garden soil and Buckalew bogs. *Fusarium oxysporium* Schlecht., from New Jersey iron and muck soils, California unfertilized, North Dakota wheat, North Dakota flax, Texas, and Colorado soils; also

by Werkenthin in Texas. *Fusarium oxysporium* var. *resupinatum* Sherb., from New Jersey orchard, iron, and Texas soils. *Fusarium lini* (?) Bolley, from New Jersey forest, Louisiana, Porto Rico, North Dakota wheat and flax soils; also by Bolley and Manns in North Dakota. *Fusarium caudatum* Wr., from New Jersey forest, Iowa, California fertilized, Porto Rico, Texas, Colorado, and Canada soils. *Fusarium* sp. (C. 60), from New Jersey meadow, Porto Rico, North Dakota wheat and flax soils. *Fusarium* sp. (H. 28), from Milltown and Buckalew bogs, New Jersey muck, Louisiana, California fertilized, Maine Aroostook loam, and Maine Aroostook infested soils. *Stysanus stemonitis* (Pers.) Corda was isolated from Texas and Maine Aroostook infested soils. *Melanconium* sp., from New Jersey garden, iron, and Sassafras loam soils. *Coniotherium Fuckelii* (?) Sacc., from New Jersey meadow soil.

Summary of Isolations

Altogether there were isolated over 200 species of fungi, 137 of which are given in Table II. The others could not be included here for lack of knowledge on the different obscure forms that have been so little studied before. The forms mentioned in this paper belong to the following 42 genera:

1. Absidia
2. Mucor
3. Zygorhynchus
4. Rhizopus
5. Saccharomyces
6. Hypoderma
7. Chætomium
8. Sordaria
9. Sphæronema
10. Monilia
11. Oidium
12. Cephalosporium
13. Populospora
14. Trichoderma
15. Aspergillus
16. Penicillium
17. Scopulariopsis
18. Sporotrichum
19. Rhinotrichum
20. Sepedonium
21. Botrytis
22. Verticillium
23. Acrostalagmus.
24. Cephalothecium
25. Acrothecium
26. Stachobotrys
27. Zygodesmus
28. Acremoniella
29. Dematium
30. Hormodendrum
31. Dicoccum
32. Basisporium
33. Cladosporium
34. Trichocladium
35. Alternaria
36. Macrosporium
37. Helminthosporium
38. Myrothecium
39. Stysanus
40. Fusarium
41. Melanconium
42. Coniothyrium

The most common genera of fungi isolated from the different soils are about the same as those mentioned in the previous paper, namely: Penicillium, Mucor, Aspergillus, Trichoderma, Fusarium, Cephalosporium, Zygorhynchus, Rhizopus, Cladosporium, Alternaria, Verticillium, and Acrostolagmus. It is also seen that a large number of these organ-

isms have been isolated by other investigators in this country and in Europe.

The question will be raised again: Is there a fungus flora in the soil? Are the fungi true soil organisms, or merely occasional visitors, limited to certain localities? Is their occurrence limited to any particular soil type, such as acid humus soils, or are they present in all soils, whether acid, neutral, or alkaline, rich or poor in humus, under different climatic conditions? The data presented in this paper warrant the conclusion that the fungi are true soil organisms. The fact that such species as *Rhizopus nigricans, Mucor racemosus, Zygorhynchus Vuilleminii, Aspergillus niger, Trichoderma Koningi, Cladosporium herbarum,* different Penicillia, Aspergilli, Fusaria, Alternaria, and others, have been isolated from different soils taken from different parts of North America and also from European soils by different investigators, would tend to show that these organisms are cosmopolitan in character and go to make up the fungus flora of the soil.

A method has been worked out (19), by which it can be shown that some fungi actually live and produce mycelium in the soil. This may prove of importance in the solving of the problem of soil fertility. Although the fungi may not occur to such an extent in the soil as do the bacteria and actinomyces, as was shown by their numbers, the fact that the spores are present in all the soils can lead us to suspect that they germinate and produce mycelium, when the moisture and temperature conditions are favorable. From the comparative data that we have on the ammonia accumulation by fungi and bacteria from organic matter (10, 18), it is seen that the fungi are very strong ammonifiers; where conditions are favorable, they will produce more ammonia out of the organic matter than bacteria do. The explanation of this lies in the difference of biochemical activities of these two groups of organisms.

The more fertile soils contain more fungi, both in numbers and species than the less fertile soils, but only a thorough investigation of the fungus content of these soils, which should cover a long period of time and include a large number of samples, could detect the difference in their fungus content. Such a difference is found in the case of the New Jersey garden, orchard, and forest soils, which are of the same soil type, but have been so modified by subsequent treatment that they are distinctly different from one another in fungus content. The forest soil had few Mucorales, but a large number of Trichodermæ and Penicillia; the orchard soil had no Trichodermæ at all and very few Penicillia, but a large number of Mucors; the garden soil which had not been brought to any extreme of treatment, and was kept up at a fairly high degree of fertility, had most of the groups of fungi well represented, without any overbalance in favor of any particular group.

No distinct difference could be observed between the influence of treatment upon the fungi of the other soils, such as the California, Maine, and North Dakota soils, probably because the organisms reported have been obtained from only one isolation; had the isolations been numerous enough, differences in the fungus flora of the soils not treated alike might have been obtained.

When the organisms isolated from the soils taken under different climatic conditions are considered, leaving out of consideration the differences in soil type and treatment, the same difficulties are encountered: the number of isolations was insufficient to allow any definite conclusions or mere general statements. To obtain definite information on that point, a large number of soils would have to be selected, representing different climatic conditions, and samples obtained at intervals of definite periods. But this would require an immense amount of work, and that was impossible under the present conditions.

But even the small amount of work done would seem to point out some distinct differences between the fungi obtained from the various soils. So, for example, the Mucorales, and also to a certain extent the Penicillia, seem to be more largely represented in the cooler climate, while the Aspergilli seem to be more abundant in the soils of the warmer climate. The Trichoderma were found extensively in the acid and water-logged soils.

The question as to whether there is a fungus flora in the soil could be answered in the affirmative on the basis of these investigations. But the question as to what is the proper fungus flora in the soil, under what conditions can it be made available for the solution of the problem of soil fertility, and also on what conditions does the presence of one or another group of fungi in the soil depend, can not be answered as yet. The difference in occurrence of the groups of fungi in the different soils is probably of a mere quantitative, rather than qualitative, character. The difference in the climatic and other soil conditions may merely modify the number of representatives of the particular group of organisms and their activities, but may not affect their total absence or presence.

Summary

1. Twenty-five soils collected under sterile conditions from different parts of North America and the Hawaiian Islands have been studied for their fungus content.

2. Over 200 species of fungi were isolated; 137 of these representing 42 genera are given in this paper.

3. The more fertile soils seem to contain more fungi, both in numbers and species, than the less fertile soils.

4. The soils of the cooler climate seem to contain a greater number of Mucorales and Penicillia, while those of the warmer climate are more

abundant in Aspergilli; this statement should not be taken in a general sense so as to cover all the soils, until more information is available.

5. The acid and water-logged soils are richer in numbers and species of Trichodermæ than normal agricultural soils.

6. Many species isolated from soils representing different parts of this country were isolated also by other investigators from different parts of Europe. This would lead one to think that there is a rather distinct fungus flora of the soil; the species occurring in any particular soil will depend on the climatic as well as on soil conditions, such as structure, acidity, moisture content, treatment, abundance of organic matter, and others.

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Literature Cited

(1) Adametz, L.
1886. Untersuchungen über die niederen Pilze der Ackerkrume. Inaug. Diss. Leipzig, 78 p., 2 pl.

(2) Beckwith, T. D.
1911. Root and culm infections of wheat by soil fungi in North Dakota. *In* Phytopathology, v. 1, p. 170-176.

(3) Chivers, A. H.
1915. A monograph on the genera Chætomium and Ascotricha. *In* Mem. Torrey Bot. Club, v. 14, p. 155-240, pl. 16-17.

(4) Dale, E.
1912. On the fungi of the soil. *In* Ann. Mycol., Bd. 10, p. 452-477, pl. 9-14.
1914. On the fungi of the soil. *In* Ann. Mycol., Bd. 12, p. 33-62, pl. 1-5.

(5) Goddard, H. M.
1913. Can fungi living in agricultural soil assimilate free nitrogen? *In* Bot. Gaz., v. 56, p. 249-305.

(6) Hagem, O.
1907. Untersuchungen über Norwegische Mucorineen, I. *In* Vidensk. Selsk., I. Math. Naturw. Klasse, Bd. 7, p. 1-50.

(7) Jensen, C. N.
1912. Fungus flora of the soil. N. Y. (Cornell) Agr. Exp. Sta. Bul. 315, p. 415-501, fig. 101-134.

(8) Lindau, G.
1907-1910. Fungi imperfecti: Hyphomycetes. Rab. Krypt. Flora Abt. 8, 9.

(9) Manns, S. F.
1903. Fungi of flax-sick soil and flax seed. Thesis. N. Dak. Agr. Exp. Sta.

(10) McLean, H. C., and Wilson, G. W.
1914. Ammonification studies with soil fungi. N. J. Agr. Exp. Sta. Bul. 270, 39 p.

(11) Namyslowski, B.
1910. Studien über Mucorineen. *In* Bul. Acad. Sci. Cracovie, Class Math. et Nat., Ser. B, p. 477-520.

(12) NAMYSLOWSKI, B.
1910. Zygorhynchus Vuilleminii, une nouvelle Mucorinée, isolé e du sol et cultivée. *In* Ann. Mycol., Bd. 8, p. 152-155.

(13) NIKITINSKY, J.
1902. Über die Zersetzung der Huminsäure durch physikalisch-chemische Agentien und durch Microörganismen. *In* Jahrb. Wiss. Bot., Bd. 37, p. 365-420.

(14) OUDEMANS, A. C., and KONING, C. J.
1902. Prodrome d'une flore mycologique, obtenue par la culture sur gelatin préparée de la pres de Russum. *In* Arch. Néerland. Sci., Exact. et Nat., t. 7, s. 2, p. 266-298.

(15) SHERBAKOFF, C. D.
1915. Fusaria of potatoes. N. Y. (Cornell) Agr. Exp. Sta. Mem. 6, p. 85-270.

(16) THOM, CH.
1910. Cultural studies of species of Penicillium. U. S. Dept. Agr. Bur. Anim. Indus. Bul. 118, 107 p.

(17) TRAAEN, A. E.
1914. Untersuchungen über Bodenpilze aus Norwegen. *In* Nyt. Magaz. Naturw. Christiania, Bd. 52, pt. 1, p. 20-121.

(18) WAKSMAN, S. A.
1916. Soil fungi and their activities. *In* Soil Sci., v. 2, p. 103-155, pl. 1-5.

(19) WAKSMAN, S. A.
1916. Do fungi actually live in the soil and produce mycelium? *In* Science, n. s., v. 44, p. 320-322.

(20) WERKENTHIN, F. C.
1916. Fungus flora of Texas soils. *In* Phytopathology, v. 6, p. 241-253.

(21) WESTLING, R.
1911. Über die grünen species der Gattung Penicillium. Inaug. Diss. Upsala. *In* Ark. Bot., Bd. 11, p. 1-156.

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